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62/76

LA-6313-PR

Progress Report

UC-48

Issued: April 1976

**Biomedical and Environmental Research Program
of the
LASL Health Division
January—December 1975**

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Contributions from the staff are indicated by group.

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**UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
CONTRACT W-7405-ENG. 36**

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Previous reports in this series, unclassified, are LA-4923-PR, LA-5227-PR, LA-5633-PR, and LA-5883-PR.

The LASL Biomedical and Environmental Research Program was supported by the USERDA Division of Biomedical and Environmental Research.

Experimental animals used in work presented in this report were maintained in animal care facilities that are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161
Price: Printed Copy \$5.00 Microfiche \$2.25

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ABSTRACT

This report summarizes research and development activities of LASL's Biomedical and Environmental Research Program for the calendar year 1975. The principal features of the program have been carried out by groups in the Health Division, supported by the Division of Biomedical and Environmental Research of the U. S. Energy Research and Development Administration. Previous annual reports of this series prepared under the auspices of the U. S. Atomic Energy Commission and the U. S. Energy Research and Development Administration are LA-4923-PR (1971), LA-5227-PR (1972), LA-5633-PR (1973), and LA-5883-PR (1974).

This report provides a summary of technical information related to the current status of projects in the major program areas of each contributing group. Sufficient detail is provided to permit the informed reader to assess the scope and significance and to obtain specific information from the publications for 1975 listed in Appendix A. Summaries useful to the casual reader desiring general information regarding current status and conclusions have been prepared by the Group Leaders and appear at the beginning of each section of the report. Investigators on the LASL Health Division staff will be happy to provide further information. Inquiries should be directed to the individuals identified throughout the report at the address shown, or they may be reached by telephone by calling 505-667-4281 or 505-667-4218.

OUTLINE OF PROGRAM

This report summarizes research and development accomplishments of the Health Division of the Los Alamos Scientific Laboratory (LASL) during the first year of operation supported by the U. S. Energy Research and Development Administration (ERDA). It represents the initial efforts to reprogram from the traditional nuclear energy focus toward the broader ERDA goals. Over the years, a strong capability in basic and applied life science has been developed, and the transition to broader energy research responsibility can be accomplished with a substantial number of applicable systems already in place. Thus, as ERDA goals for the short, intermediate, and long term emerge from the planning process, more extensive reprogramming of the LASL effort will occur, and we anticipate the appearance of a new mix of projects specifically supporting the national energy plan. Development of this new look in research will result both from initiatives of the LASL staff and from the traditional responsiveness of LASL to specific requests

from the Division of Biomedical and Environmental Research.

The Environment and Safety Program of the LASL is now being conducted by five Laboratory Divisions and spans all major funding categories of ERDA's Administrator for Environment and Safety (AES). This report deals with those aspects of the program being pursued by the Health Division. Research in the Chemistry-Nuclear Chemistry, Chemistry-Materials Science, Energy, and Medium Energy Physics Divisions is reported elsewhere. Health Division Groups involved in the RT Program are Mammalian Biology (H-4); Industrial Hygiene (H-5); Environmental Studies (H-8); Cellular and Molecular Biology (H-9); Biophysics and Instrumentation (H-10); and Organic and Biosynthesis (H-11). The table of organization for those parts of the Health Division involved in the RT Program of the AES shown below identifies the program managers of the LASL to whom preliminary inquiries regarding specific projects can be directed.

TABLE OF ORGANIZATION OF THE LASL HEALTH DIVISION (DECEMBER 1975)

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A. Mammalian Biology

Opposition to accelerated nuclear power development is based largely on concern that the potential health hazards of transuranium elements are excessive and that current health protection standards are inadequate. Because no cancer ascribable to plutonium has occurred to date in occupationally exposed populations, an attempt to estimate carcinogenicity in experimental animals has been initiated. Plutonium-bearing ceramic microspheres have been administered by both intravenous and endotracheal routes, and polonium-210 sols have been insufflated for the purpose of comparing localized "hot particles" and diffuse irradiation on lifetime tumor incidence. Additional studies involve life shortening, measurement of delayed radiation injury, and histopathological and chromosomal damage as criteria for assessing the hazards of various conditions of radiation exposure. The retention patterns for specific radionuclides in several species are now being extended to include other potential environmental toxic agents.

B. Industrial Hygiene

Inhalation is the most likely and potentially hazardous route for exposure to environmental pollutants, and developmental studies of filter efficiency, particle production characterization, and resuspension are being conducted to define proper containment conditions in the work place. To date, these studies have emphasized containment and characterization of radionuclides but provide the basis for investigating a broader spectrum of industrial pollutants. To determine the extent of plutonium exposure in man, several coordinated studies also are being conducted to measure lung burden distribution in occupationally exposed populations, the amount of plutonium in tissues from the general population, the health of LASL plutonium workers, and assessment of the health impact of plutonium in the environment.

C. Environmental Studies

The Western states and Alaska provide major energy resources and the consequent potential for major environmental impacts related to increased energy production. Ecological aspects of a variety of energy-related activities are being investigated

including the trans-Alaska pipeline, Four Corners coal utilization, uranium tailings rehabilitation, the dry geothermal energy demonstration, and radioecological surveys in northern Alaska, Los Alamos and environs, and Trinity Site. Meteorological transport models applicable to the complex terrain of the Western states are being developed using the Los Alamos vicinity as a preliminary test locale.

D. Cellular and Molecular Biology

Model DNA polymers have been employed to measure physico-chemical effects of X-irradiation and the influence of known base sequences on the transcription by RNA polymerases. These experiments allow quantitative estimates of the fidelity of transcription in the presence of physical and chemical agents. Cells in culture provide the basic system for studying radiation effects on DNA synthesis, organization of DNA in the nucleus, effects of pollutants on genetic information transfer and gene expression, nucleic acid structure, proliferation capacity, histone phosphorylation, and chromatin structure and function. Mathematical models of the immune response have been formulated, and the biochemical properties of the cell surface have been characterized. The use of flow systems to provide rapid karyotype analysis has been established for relatively simple karyotypes, and a series of cell-cycle-dependent, temperature-sensitive mutant mammalian cell lines have been derived and appear useful for cycle progression and mutagenesis studies.

E. Biophysics and Instrumentation

Rapid single-cell analysis techniques are now routine and have materially expanded the complexity of feasible kinetic experiments. New instrument improvements include multiparameter sensing, an improved light collection system, and the development of a flow-system multiangle light-scattering photometer capable of sensing viable (unfixed and unstained) cells. Parallel developments have been made in fluorescent dye evaluation and adaptation of dispersion methods to include investigation of ascites and solid tumor cell kinetics. Diagnostic applications include isolation and characterization of monocytes and evaluation of kinetic responses of tumor cells following conventional radiotherapy and exposure to negative pions.

F. Organic and Biosynthesis

Syntheses of biologically and environmentally important tracer compounds utilizing stable isotopes rather than radioisotopes permit investigations in diagnostic medicine and large-scale environmental studies impossible to conduct using radioactive counterparts. The LASL has been designated a National Stable Isotopes Resource, and an inventory of labeled compounds is available. Upon appropriate application to the Stable Isotopes Resource, compounds of interest in specific studies can be provided or synthesized.

In addition to the research activities of the Health Division Groups, an active seminar program has been maintained, and two national meetings were organized and held under the joint auspices of the Los Alamos Scientific Laboratory and the Division of Biomedical and Environmental Research.

The LASL Third Life Sciences Symposium on "Impact of Energy on Human Health" considered in detail the currently available methods for assessing impacts and drew from the scientific community, regulatory agencies, labor, and the private sector the views of each concerning not only the specific technical details but the attitudes and the concerns of industry, with the ultimate responsibility for expansion of energy production and of society forced to live with the consequences of vast technological expansion. The proceedings of this conference (held October 15-17, 1975) will be published as a Technical Information Services document within the next few months (see Appendix B for list of attendees and program).

In May 1975 a Workshop on Somatic Cell Genetics was held to describe new developments in the fields of genetics and cytogenetics, in anticipation of the recognized need to develop quantitative methods with eukaryotic systems capable of measuring toxic effects of energy-related pollutants. This workshop was an important summary of the current state of technique for selection of mutant cell lines, identification of karyologic defects, importance of chromosomal defects in somatic cells to potential malignant transformation, and finally to identification of chromosomes in various mammalian karyotypes, including man, associated with specific genes (see Appendix B for list of participants and program).

As the specific ERDA goals have emerged from conferences, planning exercises, and requirements for answers to specific energy-related questions, an extensive second round of reprogramming has occurred in projects specifically able to respond. Thus, the next annual report will reflect different priorities and different descriptions of effort. Because many of the systems reported here adapt readily to toxicology associated with the work place, sufficient continuity in titles will remain to permit the reader to follow the transition from the traditional nuclear focus to the newer projects. To translate planning into productive bench science is the immediate goal of the LASL Health Division. To use the results of that effort for the longer term assessment of health, environmental, social, and economic impacts of accelerated energy production is our eventual target.

Donald F. Petersen
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MAMMALIAN BIOLOGY GROUP (H-4)

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INTRODUCTION

The primary efforts of this group are geared to determining the toxicity of various agents and their potential insults to mammalian systems. The agents studied presently are both nuclear and non-nuclear in nature, with emphasis clearly shifting toward the latter. End points of the studies vary from gross observations such as survival time to more mechanistically oriented, detailed investigations that are measured at the microscopic level. There are five major research thrusts within the group, four being funded from the ERDA Division of Biomedical and Environmental Research.

During the past 15 to 20 yr, there has been an active effort to collect data suitable for use in establishing radiation protection guides. Soluble forms of radionuclides at the tracer level have been

administered by various routes to several species of animals, and the retention, tissue distribution, and excretion patterns have been measured with time. In addition to single parenteral and intragastric administration, studies have also been carried out to investigate the metabolic behavior of an element when placed daily in the drinking water. The species used have included mostly mice, rats, monkeys, and dogs. Using body weight as a denominator, extrapolations to standard man have been made. The data from these studies have been used successfully by such bodies as the National Council on Radiation Protection and Measurements (NCRP) and the International Commission on Radiological Protection (ICRP) in setting regulatory standards for the nuclear industry worker and for the population as a whole. The current trend in this effort is toward

studying the effect of administering various dosage levels of a potential toxicant to detect and measure possible alterations in the normal non-toxic level metabolic patterns for the agent under investigation.

An external irradiation project has been part of the Mammalian Biology Group's activities for a number of years. In the past, many species have been exposed to either x rays or gamma rays from cobalt-60, with the primary goal being determination of life-span shortening and certain gross pathological changes such as those associated with the hemopoietic system. Emphasis has been shifted recently from the larger species such as the monkey and dog to the smaller laboratory rodent. Bringing together in one laboratory, under identical exposure and housing conditions, and utilizing the abilities of one team of personnel, a study is in progress that will observe the long-term biological effects in mice as a function of dose rate, total dose, and age at exposure. Chromosomal changes are being studied, both for gross aberrations and for specific sites of damage through banding changes. The range in parameters under study is wide, and the life-span data should allow for extrapolation to very low-dosage regions. The determinations of a linear or non-linear relationship between these parameters should add greatly to our present views on the effects of very low-level irradiation on mammalian systems.

A third major effort deals with the so-called "hot particle" problem. Ceramic (ZrO_2) plutonium-containing microspheres have been placed into lung capillaries via the jugular vein of Syrian golden hamsters. A precise determination of the numbers of

spheres and their specific activity has enabled a rather unique estimate of the localized radiation dose to surrounding lung tissue. Tumor incidence has been the primary end point in these studies, with the objective of determining the relative hazard of localized vs diffuse alpha irradiation as the fraction of lung irradiated is varied from less than 0.01 to almost 1.0. Additional insults have also been used in conjunction with alpha particle radiation to determine if synergistic reactions can occur, as measured by tumor incidence. This project continues to supply valuable information directly related to the evaluation of hazards associated with the nuclear power program.

Immunological assessment of the mechanisms of the etiology of cancer induction is a newly begun endeavor. Techniques for assessing immunocompetence are being incorporated into the laboratory procedure so that a model can be established to assess the action of the immune system to various potential carcinogens associated with the development of non-nuclear energy sources such as coal gasification. Since this project is in its infancy, no detailed information will be provided in this report.

There is a small effort within the Group to help assess the mammalian radiobiological effects of high-LET radiation produced by the accelerator at the Clinton P. Anderson Meson Physics Facility (LAMPF). The funding for this is from the National Cancer Institute through the University of New Mexico Cancer Research and Treatment Center. The findings related to this project are not included herein.

EFFECTS OF INTERNAL RADIATION ON LIVING ORGANISMS
(HOT PARTICLE PROGRAM)

[E. C. Anderson, L. M. Holland, J. R. Prince, D. M. Smith, S. G. Carpenter, G. A. Drake, J. E. London, J. S. Wilson, and R. G. Thomas]

Introduction

There is current controversy as to whether intense, localized irradiation of a small volume of tissue is more or less harmful than the same total energy delivered diffusely to a large volume. In particular, the contention has been made that presently recommended safety limits for inhaled PuO_2 are far too high. The common foundation of several such contentions is the assumption that tumor incidence, measured when large volumes of tissue are irradiated, can be applied to small volumes which may receive large radiation doses from high local concentrations of PuO_2 . Experiments were begun at LASL in 1971 to test this basic assumption, and four different modes of exposing the lung to alpha radiation have been used to date: intravenous (IV) microspheres, intratracheal (IT) microspheres, IT colloidal sols, and inhaled (INH) aerosols. Intravenous microspheres irradiate the lung locally by completely static particles; IT microspheres are partially mobile, but only the lung is significantly irradiated; IT colloidal sols are extensively distributed throughout the entire lung, clear the pulmonary system rapidly, and thus irradiate several other organs; and INH aerosols share some of the properties of the IT sol exposures but also provide a more practical exposure condition to either mono- or polydispersed particles. A comparative measurement of the tumorigenicity of a given quantity of alpha activity administered by these routes may provide a direct answer to the question of relative hazard and an indication of the nature of the significant dose-distribution factors.

Our results so far indicate that individual microspheres produce a strikingly low degree of damage relative to that resulting from exposures to either aerosols or colloidal sols for a number of end points including life shortening, general pathology, and tumor incidence.¹⁻⁸ The immediate objective is understanding this difference and identification of the factors which are missing from the microsphere exposures.

Tumorigenicity of Intravenous Microspheres

In the intravenous mode of exposure, uniform spheres (10 μm in diameter) are injected into the jugular vein of Syrian hamsters (which are known to be susceptible to lung tumor induction) and lodge quantitatively and permanently in the capillary bed of the lung. Thus, an accurately known alpha radiation dose is delivered to well-defined regions of the lung which are marked by the continued presence of the microscopically visible spheres. This experiment is particularly well adapted to the study of the effects of isolated particles, as well as of results of increasing the number of spheres to produce interactions. The spheres lodge throughout the capillary bed of the lung; thus, examples of exposure of all lung components (from the bronchi to the alveoli) can be found.

To date, over 2500 hamsters have been exposed to more than 200 000 000 microspheres by intravenous injection. Three parameters have been varied, as shown in Fig. 1. Here the abscissa is the specific activity of the spheres, which was varied from 0.016 to 59 μCi per sphere; the ordinate is lung burden, which varied from 0.14 to 700 nCi ; and the diagonal lines are the number of spheres administered, which varied from 2000 to 1 000 000 per animal. The

Fig. 1. Distribution of intravenous microsphere experiments and observed lung tumors (see text for explanations).

points represent experiments, the triangles showing those in which tumors have been observed. The pair of numbers beside each point give (the number of tumors observed)/(the number of animals completely examined). Where no numbers are given *beside the point*, an experiment (50-100 animals) is in progress but no results are yet available because *microscopic examination and data processing are incomplete*.

The data include all tumors identified by October 1975. Two types of experiments are included: those which are "complete" (i.e., all animals are dead and all microscopic histological examination has been done) and those which are represented in the necropsy material for the first 9 months of 1975. All of the latter material has been examined and provides a sampling of current experiments. The sampling may not be without bias as experiments begun at different times will contribute animals with different durations of exposure.

The previously reported observation of three tumors in the 2000- to 6000-microsphere exposures was raised to five by the identification of a benign adenoma and a frankly malignant mucinous adenocarcinoma during the past summer. However, the discovery of a mucinous adenoma in control groups totaling 236 hamsters raises the question as to how much these incidences exceed the background rate of "spontaneous" tumors. At the other end of the experimental range, where very large numbers of

microspheres (10^5 per animal) result in a wide distribution of energy deposition somewhat resembling the diffuse case, preliminary results suggest that tumor incidence may be higher. Seven tumors, of which five were adenomas, have been identified in these exposure groups. The 12 tumors so far observed for all experiments (Table I) are distributed through a total of 1240 hamsters which have been completely examined histologically.

It is still too early for a definitive conclusion regarding the dependence of tumor induction on the three variables, but it appears significant that nine out of the 12 tumors occurred at lung burdens exceeding 60 nCi in 326 hamsters. The remaining three tumors occurred in 914 hamsters exposed to lower doses (see Table II). At the high lung burdens, there is a suggestion that tumor incidence is higher when the exposure is to large numbers of lower specific activity spheres. Thus, the three experiments with 60 000 to 80 000 spheres and 100 to 200 nCi lung burden gave five tumors in 69 hamsters, while the two experiments with 59-pCi spheres at 118 and 354 nCi gave only two tumors in 220 animals. These results are in accord with the expectation that, at high concentrations of microspheres, tumor incidence may rise because the resulting irradiation pattern will begin to approach a diffuse exposure pattern within the lung.

TABLE I
TUMORS OBSERVED IN INTRAVENOUS MICROSPHERE EXPOSURES

Lung Burden (nCi)	Number of Spheres	Specific Activity of Spheres (pCi per sphere)	Duration of Exposure (wk)	Type of Tumor
354	6000	59	104	{ Mucinous adenocarcinoma
			115	
168	400 000	0.42	81	Adenoma
	80 000	2.1	100	Adenoma
126	60 000	2.1	46	{ Mucinous adenocarcinoma
			58	
			61	
			64	
70	1 000 000	0.07	136	Mixed squamous-adenocarcinoma
4.2	2000	2.1	52	Undifferentiated sarcoma
0.84	2000	0.42	41	{ Hemangiosarcoma
			62	
				Adenoma

TABLE II

DISTRIBUTION OF TUMORS OBSERVED IN IV
MICROSPHERE EXPOSURES (AS OF OCTOBER 1975)

Lung Burden (nCi)	Number of Tumors	Number of Hamsters Examined
200-600	2	148
60-200	7	178
20-60	0	293
6-20	0	172
2-6	1	150
0.6-2.0	2	160
0.1-0.6	0	139
Total	12	1240

The tumor incidence remains far below that predicted by "hot particle" models which assume that isolated, single particles are tumorigenic. If the dependence of tumor frequency on total dose and sphere number described above is confirmed, then the majority of the tumors which we have seen may be ascribable to the cooperative action of large numbers of particles which together irradiate a significant fraction of the lung. The maximum tumor incidence we have observed is also far below the 50 to 90% incidence reported by Little for exposure to comparable amounts of colloidal polonium-210. Our results, therefore, do not support the contention that localized irradiation of the lung is more tumorigenic than diffuse exposure.

Tumorigenicity of Intratracheal Microspheres

In the intratracheal mode of exposure, the microspheres are instilled deep into the lung using a pulsed dental hygiene water jet. Two exposures were made: in March 1974, 62 hamsters were exposed to 36 000 spheres of specific activity 2.1 pCi per

sphere, and in October 1974, 171 hamsters were exposed to 30 000 spheres at 1.1 pCi per sphere. Lung burdens were 76 and 33 nCi, respectively; a variety of Fe_2O_3 instillations were given to some subgroups. Whole-body retention has been followed for hamsters in the second group for periods of up to 300 days (by *in vivo* gamma counting of the cobalt-57 tag added to the spheres). At 200 days, the average retention for 27 animals was $92 \pm 3\%$, corresponding to a biological half time of greater than 900 days. It is not known at this time to what extent the spheres may move within the lung.

Of these animals, 69 came to post during the first nine months of 1975, and microscopic pathology has been completed. Six tumors (all adenomas) were found, as summarized in Table III. While it is too early for definitive conclusions, preliminary indications are that the tumor incidence is not unlike that observed for the intravenous microspheres at somewhat higher lung burdens and sphere numbers.

Tumorigenicity of Intratracheal Polonium-210 Soils

Experiments using colloidal polonium-210 soils delivered intratracheally to hamsters were initiated for comparison of the microsphere exposures with a diffuse irradiation from which generalized lung damage and a high tumor yield have been reported.⁹ The methods and some results have been reported previously.^{3,4,7} We summarize here the results of the first experiment for which the pathology is complete.

In this experiment, each hamster received an intratracheal instillation of 54 nCi of colloidal polonium-210 once a week for 15 wk. Of 18 animals surviving beyond 19 wk after the initial dose, most suffered a type of general lung damage usually associated with radiation pneumonitis (i.e., septal

TABLE III

TUMORS OBSERVED IN IV MICROSPHERE EXPOSURES

Lung Burden (nCi)	Number of Spheres	Specific Activity of Spheres (pCi per sphere)	Duration of Exposure (wk)	Type of Tumor
76	36 000	2.1	50	{ Mucinous adenoma
			52	
33	30 000	1.1	25	{ Adenoma (two)
			27	
			29	
			39	
				{ Adenoma (many)
				{ Adenoma

thickening, moderate fibrosis, and increased macrophage activity) (see following section). One benign and three malignant tumors occurred in four hamsters. Of the malignant tumors, two were mucinous adenocarcinomas which occurred at 32 and 49 wk, and the other was a mixed squamous-adenocarcinoma found after 57 wk. The fourth animal died as a result of a large adenoma of the lung after 57 wk. One hamster is still alive after 84 wk. In our hands, the efficiency of tumor production by polonium-210 appears lower than that reported by Little *et al.*⁹ However, when the smaller number of animals and the difference in exposure (54 nCi x 15 wk, LASL; 200 nCi x 7 wk, Harvard) are considered, the difference in tumor incidence (24% vs 50%) is probably not significant. For comparison with the microsphere exposures, the polonium-210 exposure to 54 nCi for 15 wk (with a biological half time in the lung of about 1 wk) delivers approximately the same amount of energy to the lung as would 15 to 20 nCi of plutonium as microspheres in the first year of exposure. As reported above, it appears that only with microsphere lung burdens of the order of 100 nCi distributed in a very large number of low specific activity microspheres does the tumor incidence reach 10%.

Additional exposures are now in progress using larger numbers of hamsters exposed to a graded series of doses of both polonium-210 and plutonium-239 administered intratracheally as colloidal sols. These experiments will provide quantitative dose-response curves and may help identify the factors responsible for the high tumor induction by colloidal alpha activity.

The Pathology of Focal and Diffuse Irradiation of the Lung

One of the important observations of the "hot particle" program is the apparent lack of damage to lung tissue from individual static microspheres even when the alpha dose to surrounding tissue is high. As long as the biologically inert microspheres remain in the capillaries, they usually do not elicit any inflammatory reaction (Fig. 2). Only rarely, when the microspheres are extruded into an alveolar space, is there a foreign body reaction consisting of focal accumulations of macrophages (Fig. 3). Osseous metaplasia and focal accumulation of hemosiderocytes have been seen to a low

Fig. 2. Microsphere showing no reaction.

Fig. 3. Extruded microsphere with macrophages.

degree in both control and experimental animals. Bronchiolar adenomatoid lesions (BAL) (Fig. 4) have been observed in experimental animals, particularly at long post-injection times, but here too the frequency is relatively low. There has been no histological evidence of pneumonitis or fibrosis in the IV microsphere animals where histological examination is complete except that, in some animals where the local concentration of microspheres is high, mild focal fibrosis has been observed. Under these conditions, mechanical occlusion of portions of the capillary bed is thought to be a contributing factor.

By comparison, when alpha-emitting polonium-210 is administered intratracheally and the irradiation to the lung is more diffuse, the lung damage is more severe and generalized. In addition to an increase in osseous metaplasia, focal hemosiderosis, focal pneumonia, and BAL, there was edema and thickening of the intra-alveolar septa and moderate fibrosis even at relatively early post-exposure times (Fig. 5). Increased macrophage-septal cell activity was observed in all of the polonium-210 animals examined.

There is a marked difference histologically between the effect of a static foci of irradiation involving discrete cell volumes and that of a diffuse general irradiation of the lung.

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Fig. 4. Minimum bronchiolar adenomatoid lesions (BAL).

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EFFECTS OF IONIZING RADIATION FROM EXTERNAL SOURCES
ON LIVING ORGANISMS

[J. F. Spalding, J. E. London, O. S. Johnson, and
J. R. Prine]

Delayed Radiation Injury from Partial-Body X-Ray
Exposures

The nature and magnitude of radiation-provoked injury that results in premature death and/or a physiological decrement in resistance to biological stress are being studied. In this experiment, general areas of the body of two mouse strains were subjected to x rays, and the comparative nature of the resulting injury is being measured by a decrement in resistance to continuous gamma-ray stress or a reduction in life span. The techniques used to make selective *in situ* body area x-ray exposures are shown in Fig. 1.

Eleven groups of each strain (C57BL/6J and RF/J), 24 mice per group, were treated as shown in Table I. The x-ray doses were given at 200 rads per min in 200-rad fractions at 7-day intervals and programmed so that the last fraction in each group was given on the same day. Twenty-eight days after

Fig. 1. Restraining device used for partial-body shielding during x-ray exposure: (A) and (B) restraint position for whole-body exposure while anesthetized; (C) and (D) restraint position for gut exposure and gut shielding, respectively, using 1/4 in. of lead.

TABLE I
EXPERIMENTAL PROTOCOL FOR RADIATION
EXPOSURES TO MICE

<u>Group</u>	<u>Treatment</u>
<u>No Anesthesia</u>	
1	0 dose Control
<u>Anesthetized^a</u>	
2	0 dose Control
3	1200 rads Whole-body
4	800 rads Whole-body
5	400 rads Whole-body
6	1200 rads Gut-exposed
7	800 rads Gut-exposed
8	400 rads Gut-exposed
9	1200 rads Gut-shielded
10	800 rads Gut-shielded
11	400 rads Gut-shielded

^aAll mice were anesthetized for the same number of exposures or sham exposures.

the last conditioning exposure, all groups were challenged to death in a 50-rad per day cobalt-60 gamma-ray field. In addition, a replication with the two strains of mice and radiation treatments was done to observe the life-shortening effects and cause of death. Mice were bled for routine hematology and weighed at various times throughout. The mice were necropsied at death to determine the cause.

The data on mean after-survival (MAS) times have been compiled but have not been completely evaluated. Necropsies and histopathological examinations of animals which succumbed during the invariant gamma-ray challenge showed that (almost without exception) the mice in all treatment groups (Table I) died because of hemopoietic failure. The delayed radiation injury from the different conditioning treatments has been expressed as percentage reductions in MAS in Table II. Whole-body, gut-exposed, and gut-shielded groups all showed delayed radiation injury that increased with total conditioning dose. The reduction in MAS per 100 rads of x-ray conditioning dose was greatest for the whole-body conditioned and least for the gut-exposed mice (Table II). Both mouse strains responded similarly to the MAS method of measuring delayed radiation injury. When the mean reduction in MAS per 100-rad

TABLE II
REDUCTION IN MEAN AFTER-SURVIVAL TIME DURING INVARIANT GAMMA-RAY STRESS (5 rads per h)
EXPRESSED AS PERCENTAGE VALUES

Group Number	Conditioning Treatment		Percent Reduction in MAS	
			Total	Per 100-Rad Conditioning Dose
<u>RF/J Strain</u>				
1	0 dose	No anesthesia	(18.1) ^a	--
2	0 dose	Anesthetized	Control	--
3	1200 rads	Whole-body	57.8	4.82
4	800 rads	Whole-body	44.8	5.60
5	400 rads	Whole-body	16.9	4.23
6	1200 rads	Gut-exposed	12.9	1.08
7	800 rads	Gut-exposed	10.4	1.03
8	400 rads	Gut-exposed	3.4	0.85
9	1200 rads	Gut-shielded	40.0	3.33
10	800 rads	Gut-shielded	26.0	3.25
11	400 rads	Gut-shielded	20.2	5.05
<u>C57BL/6J Strain</u>				
1	0 dose	No anesthesia	12.5	--
2	0 dose	Anesthetized	Control	--
3	1200 rads	Whole-body	74.0	6.17
4	800 rads	Whole-body	49.0	6.13
5	400 rads	Whole-body	34.3	8.58
6	1200 rads	Gut-exposed	16.6	1.38
7	800 rads	Gut-exposed	14.1	1.76
8	400 rads	Gut-exposed	1.4	0.35
9	1200 rads	Gut-shielded	41.3	3.44
10	800 rads	Gut-shielded	23.3	2.91
11	400 rads	Gut-shielded	13.2	3.30

^aThe number in parentheses indicates a MAS greater than the 0 dose to the anesthetized control.

conditioning dose of the three gut-exposed groups (6, 7, and 8) is added to the mean value of the three gut-shielded groups (9, 10, and 11), the sum is of the same general magnitude as the mean reduction in MAS per 100-rad conditioning dose of the three whole-body exposed groups (3, 4, and 5) of the RF/J strain but not in the C57BL/6J strain. Gut exposures included 25% of animal total body weight, and gut-shielded exposures (exposing 100% of the bone tissue) included 75% of the total body weight. If percent reduction in MAS per 100 rads of conditioning exposure in Table II is adjusted for the body weight exposed, delayed injury from gut-exposed and gut-shielded groups in both strains is similar. This very gross implication is contrary to the generally

accepted view that bone tissue is the critical organ in long-term injury from radiation exposure.

The life-shortening phase of this project has not yielded enough data to determine whether these two end points (life-shortening and MAS during gamma-ray stress) will measure similar or completely different delayed effects of radiation injury. The average weight gain (or loss) during conditioning exposures, recovery, and 50 days of continuous gamma-ray challenge for the whole-body conditioned groups of the C57BL/6J strain are shown in Fig. 2. Weight gain was inversely related to the total conditioning dose. The short-term effects of the x-ray exposures were apparently overcome during the 28 days of recovery. However, the rate of weight

Fig. 2. Mean weight gains (or losses) of whole-body x-ray conditioned C57BL/6J strain mice during x-ray conditioning, recovery, and 50 days of gamma-ray challenge exposure.

loss during the gamma-ray challenge exposure was directly related to the conditioning dose, reflecting a residual radiation lesion in the metabolic process of gain or retention of body weight.

Life Shortening, Histopathological and Chromosomal Damage as Criteria for Assessment of the Impact of Various Irradiation Conditions in the Mouse

There is insufficient information on the biological effects of the all-encompassing combination of variables (doses, dose rate, and age at exposure) used in this radiation study to assess properly the impact of external gamma-ray exposure of a population to above-natural environmental levels. Male mice are being exposed to cobalt-60 gamma rays in groups of approximately 25 each, with six dose rates and total doses, at ages varying in four steps from newborn to 15 months. These individual parameters are shown in Table III. The dose rates to each

group are provided by placing the animals in their cages at properly selected distances from the source. Two sources are necessary to attain the wide range in doses and dose rates indicated (Table III). Animals are checked at least twice a day, 7 days/wk, to minimize autolysis following death.

Life span and cause of death are major experimental criteria, and dead animals will be preserved for histopathological examination. Selected series of animals are assigned for serial sacrifice by blood withdrawal to observe chromosomal aberrations. The techniques used for fixing the chromosomes require approximately 0.1 ml of cardiac blood, or about 10^6 leukocytes. The medium consists of newborn calf serum, L-glutamine, calcium chloride, and phyto-hemagglutinin, with commercial medium (RPMI-1640) comprising the largest proportion (94%). After incubation at 38°C, Colcemid is added for 4 h, the nuclei are swollen with KCl, and after fixation with alcohol and acetic acid, the metaphase spreads are made on ice-cold slides. It is anticipated that at least three replications of the experimental plan will be done (100 animals/point) in tandem. The first replication was started on September 9, 1975. There have been no late-effect deaths to this date; however, preliminary chromosome spreads have shown some breaks and other abnormalities at total doses as low as 20 rads. Control mice have shown no such damage. Although the extrapolation from mouse to man is always questionable, in this instance, the approach appears to be the only logical one for the results desired. The doses and dose rates chosen span the range of significant importance to any anticipated real-life exposures. The ages of the mice chosen represent human chronological separations that would extrapolate from the infant to advanced age. The results of this experimentation will assist considerably in evaluating our future plans where nuclear energy will be involved.

TABLE III

REGIME FOR EXPOSURE OF MICE TO COBALT-60 GAMMA RAYS^a

Total Dose (rads)	0.7	2.1	Dose Rate (rads/day)		56.7	Dose Rate (rads/min)	
			6.3	18.9		25.0	Exposure Time (min)
			Exposure Time (h)				
20	686	229	76	25	8.5	0.8	
60	2057	686	229	76	25	2.4	
180	6171	2057	686	229	76	7.2	
540		6171	2057	686	229	21.6	
1620			6171	2057	686		

^a Ages of exposure are newborn, 2, 6, and 15 months; approximately 25 mice per replication.

[J. E. Furchner, R. G. Thomas, G. A. Drake, J. E. London, and J. S. Wilson]

Retention Patterns of Potential Environmental Toxicants

Selenium, cobalt, tin, and cadmium retention patterns have been studied in various mammalian species. Mice, rats, monkeys, and dogs were chosen because of their prominence in laboratory studies and because they represent a wide range in body weights to use as an extrapolative means to the human that might be exposed to one of these potential toxicants. Whole-body retention patterns were determined from measurement of the gamma rays emitted by a suitable radioisotope of the element under investigation. Tissue distribution patterns of the radionuclide were also determined through serial sacrifice and necropsy at various intervals after administration. Excreta collections for the radionuclide determination were made in most instances.

Selenium-75 as the selenite was administered by oral (IG) and intravenous (IV) routes to all four species. Intraperitoneal (IP) injections were made into mice and rats. More than 90% was absorbed from the gut in all species but monkeys, where much less was absorbed. Whole-body retention was described by the sum of three exponentials in all species, whatever the route of administration. Excretion of selenium-75 favored the urinary route by about a factor of three after the first few days of administration. The equilibrium body burden (EF) during chronic ingestion was calculated by integrating the retention functions over an infinite period of intake. When the logarithms of these EF values were plotted against the logarithm of body weight, the results shown in Fig. 1 were obtained. Extrapolation of these whole-body data to a 70-kg man led to the conclusion that the present Radiation Protection Guide (RPG) perhaps should be reduced by a factor of five. Liver and kidney appear to be the target organs for selenium, as they concentrate the isotope to the greatest extent. Calculations based upon their relationships to body burden and long-term half-life of retention indicate that the present RPG is perhaps a factor of 10 too high.

Fig. 1. Equilibrium factor (time-integral of retention function) for IG animals as a function of body weight. Extrapolation to 70 kg (X) gives a value of 30. The upper point at 70 kg is the value for man injected intravenously with selenomethionine.¹

The behavior of cobalt was rather unconventional. The long-term half-life of retention in mice and rats was longer (300 to 400 days) than in monkeys and dogs (150 to 200 days). Past results with most substances such as selenium have normally shown a longer relative retention with increasingly larger species. We feel that part of the explanation of the seeming anomaly lies in the differences in vitamin B₁₂ metabolism between the species, and it is known that rodents often resort to coprophagy to attain their daily requirements. Through this means, the cobalt itself may also be recycled. There is also some possibility that the unusual retention patterns may be the result of a carrier effect. Approximately the same amount of cobalt was administered to each species, resulting in a very much larger concentration ($\mu\text{g per kg}$) in rodents. This possible carrier effect is currently under investigation in our Laboratory. Data obtained from daily administration of cobalt-60 in the drinking water of rats indicate that absorption to the blood from the gut is approximately 10% of the dose. Our short-term studies favor the liver and kidney as the tissues with largest concentrations. A few long-term (460 days) rats following intravenous administration indicate that bone may be the organ of greatest concentration. Future studies should resolve this question. EF values were not meaningful in extrapolation to humans because of the unusual pattern in whole-body retention characteristics.

Tin, as tin-113, was injected by the IG, IP, and IV routes into mice, rats, and African white-tailed rats and by the IG and IV routes in monkeys

and dogs. Less than 5% was absorbed from the gut in all cases. There was much similarity in the retention patterns following parenteral administration; excretion of tin-113 following parenteral injection considerably favored the urinary route. Because of the similarity in retention patterns between species, a plot of log EF values vs log body weight was essentially a constant. Thus, it is assumed that the human would behave similarly to other species in this regard. Bone was the chief site of tin deposition and should be considered the target organ upon which to base RPG values. The RPG values set by the ICRP in 1960 appear to be adequate in view of the data presented.

Data on cadmium retention in rodents, as measured with cadmium-109 tracer, are currently being analyzed. In monkeys and dogs, retention after IV injection of tracer quantities is extremely long, showing no detectable decrease over a one-year period. Absorption from the gut is less than 10%, but that which enters the blood remains internally bound for a long period of time. Most of this, as observed from preliminary results, appears to be

localized in the kidney and liver. One dog has received oral doses of stable cadmium following the single IV administration of tracer-level cadmium-109. It has required a dosage approaching 50 mg per day to bring about any toxic effects, but the study is continuing and routine physicals are being performed to enable a recording of any adverse changes in physiological behavior. Data from these experiments are insufficiently analyzed at present to allow comment on current RPG values and extrapolations to humans.

With elements such as discussed here, studies will continue to determine the effect of potential toxic levels of the stable elements upon normal tracer-level retention patterns. The inhalation route will also be introduced in future studies to simulate more nearly the manner in which exposures to atmospheric pollutants are most likely to occur.

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INTRODUCTION

The Division of Biomedical and Environmental Research funds several projects in the Industrial Hygiene Group (H-5) related to the evaluation and control of health hazards associated with the working environment. These include applied studies on the sampling, characterization, and control of fine particles and the analyses of tissues from autopsies of individuals from the general public and those potentially exposed to plutonium.

Primary efforts in the applied aerosol program have been directed toward evaluating the performance of filtration systems used in air cleaning engineering controls for facilities handling plutonium. Experimental work with plutonium aerosols has been completed, and additional studies involving ultra-fine aerosols smaller than 0.2- μ m aerodynamic diameter will use other high-density materials. Studies are continuing to quantitate the effects of saltation on particle resuspension and to provide a basis for extending preliminary controlled laboratory

experiments to simulate environmental conditions of concern. In cooperation with the Mammalian Biology Group (H-4), test aerosols and exposure chambers have been developed for small-animal inhalation exposure experiments.

The analysis of autopsy tissue samples for plutonium has continued. Samples from individuals in the general population exposed only to worldwide fallout are providing base-line data from several geographic locations in the United States. Statistical analyses have compared these nationwide base-line levels with one area where a major plutonium facility operates. Detailed chemical analyses of samples from two occupationally exposed individuals indicate the levels of exposure associated with these individuals, the concentration and size distribution of plutonium within the lung, and the absence of histological changes. Both the general population and occupational exposure samples have defined those organs having the highest concentration and largest amount of plutonium.

FINE PARTICLE STUDIES RELATED TO HEALTH AND AIR
CLEANING REQUIREMENTS FOR ENERGY SYSTEMS

Evaluation of HEPA Filter Collection Efficiency for
Fine Particles

[M. I. Tillery, J. C. Elder, C. I. Fairchild, M.
Gonzales, and H. J. Ettinger]

Widespread use of HEPA (high-efficiency particulate air) filters for air cleaning in existing nuclear facilities and potential use of these filters in new facilities (i.e., reactor fuel processing) require performance information with respect to potentially toxic fine particles. Performance of these filters for larger respirable particles primarily collected by inertial mechanisms is well established.¹ Collection efficiency for submicron particles collected predominantly by diffusion (diameter $\leq 0.3 \mu\text{m}$ for unit density particles) is not as well established. In addition, the enhanced collection of high-density particulates due to overlapping of inertial and diffusional collection mechanisms needs to be quantitated because of the potential toxicity of submicron particles.

Initial work with high-density particles has been carried out with polydispersed aerosols of ²³⁸PuO₂ having a density of approximately 10. Using a mechanical grinding process, aerosols having activity median aerodynamic diameters (amad) as small as 0.22 μm have been provided. Results of this study indicate that the plutonium oxide particle size of maximum penetration is between 0.43 and 0.65- μm aerodynamic diameter (D_{ae}), compared to the generally accepted value of 0.3 μm for unit density particles.² Results also indicate that HEPA filter penetration at the most penetrating particle size is less for high-density particles than for low-density particles typically used for filter testing.

Results of full flow tests against aerosols of plutonium dioxide are given in Table I. The results are classified into two groups, depending on quality control test results using monodispersed dioctyl phthalate (DOP) aerosols of 0.3- μm diameter. The low-penetration filters had quality control DOP penetrations ranging from 20 to 150 $\times 10^{-6}$, while the high-penetration filters ranged from 151 to 300 $\times 10^{-6}$. The average penetration for polydispersed plutonium oxide aerosols was one-fifth to one-sixth

TABLE I

HEPA FILTER PERFORMANCE AGAINST
DOP AND PLUTONIUM OXIDE AEROSOLS

	Mean	Standard Deviation
<u>DOP (Quality Control) Penetration</u>		
Low penetration	50 $\times 10^{-6}$	$\pm 22 \times 10^{-6}$
High penetration	220 $\times 10^{-6}$	$\pm 30 \times 10^{-6}$
<u>Plutonium Oxide Penetration</u>		
Low penetration	8.3 $\times 10^{-6}$	$\pm 7.1 \times 10^{-6}$
High penetration	43 $\times 10^{-6}$	$\pm 43 \times 10^{-6}$

the 0.3- μm DOP penetration. Penetration for plutonium oxide aerosols in the maximum penetration size range was 28 $\times 10^{-6}$, approximately half the DOP penetration for filters in the low-penetration group. These results show that the standard quality control procedures using DOP provide a conservative estimate of HEPA filter performance against plutonium aerosols. This completes the experimental work with plutonium aerosol, and additional work in progress will involve high density metal aerosols smaller than 0.2 μm .

Production of aerosols having median diameters smaller than 0.22 μm requires different generation techniques. Initial work with dilute hydrosols indicates that this process will provide small particle aerosols of sufficient concentrations to test one HEPA filter. A new system will be used to measure the collection efficiency for particles smaller than 0.2 μm amad having densities ranging from approximately 1 to 18 g/cm³. Particle size measurements in the submicron range will be made with a low-pressure cascade impactor (inlet pressure $\sim 60 \text{ mm Hg}$) which has been constructed and is being calibrated. A 24-jet compressed air nebulizer has been constructed for aerosolization of the hydrosol suspension and is being evaluated with respect to output, particle size, and solution evaporation. Particle size measurements are being made currently with a commercial Andersen 7-stage cascade impactor operated at $\sim 85 \text{ liters/min}$, separating particles down to an aerodynamic diameter of 0.12 μm . Upon completion of low-pressure impactor calibration and generator evaluation, HEPA filtration efficiency will be measured using

ultrafine aerosols of high-density metal oxides tagged to permit analysis by neutron activation.

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Resuspension of Particles

Prediction of transport and migration of contaminant material requires an understanding of the parameters that determine particle resuspension. The wide variability encountered in environmental resuspension measurements indicates that the relative importance of the many parameters which contribute to resuspension is not adequately understood. In an effort to isolate, eliminate, or control some of these parameters and to determine which are of primary importance, resuspension experiments are being conducted in a wind tunnel where the effect of saltating particles (diameter $> 80 \mu\text{m}$) on resuspension of smaller particles (diameter $< 10 \mu\text{m}$) can be studied. The resuspension surface consists of a stainless steel plate with a roughness height of $3.4 \mu\text{m}$. Preliminary experiments involve covering the resuspension surface with particles and rapidly establishing steady-state air flow conditions within the tunnel. Saltation particles are introduced into the wind tunnel 3 m upstream of the resuspension surface.

Resuspension studies have been carried out using two sizes of small bed particles together with (a) no saltation particles; (b) $100\text{-}\mu\text{m}$ -diameter saltation particles; and (c) $200\text{-}\mu\text{m}$ -diameter saltation particles. These two different sizes of saltation particles are introduced in equal numbers and equal masses in separate experiments. Small bed particles are of spherical aluminum, having log-normal size distributions with count median diameters (CMD) of 3.7 and $7.0 \mu\text{m}$ and geometric standard deviations (σ_g) of 1.7 and 1.6 , respectively. The saltation particles are monodispersed glass spheres. Resuspension rates, defined as fraction of initial mass resuspended per second, increased in the order of no saltation (condition A): $100\text{-}\mu\text{m}$ -diameter saltation particles (condition B); and

$200\text{-}\mu\text{m}$ -diameter saltation particles (condition C) when measured at the same wind velocities and with equal numbers of saltating particles. Equal masses of saltating particles (8 times as many $100\text{-}\mu\text{m}$ -diameter particles compared to $200\text{-}\mu\text{m}$ -diameter particles) produced higher resuspension rates with $100\text{-}\mu\text{m}$ -diameter saltation particles, indicating that resuspension is proportional to momentum of individual impacts on the bed particles and number of impacts. Similar patterns of increase in resuspension rate were observed for both 3.7- and $7.0\text{-}\mu\text{m}$ -size bed particles; however, the effect of saltating particles was considerably greater on resuspension rates for smaller size bed particles. Except for tests using $200\text{-}\mu\text{m}$ saltation particles, resuspension rates for $3.7\text{-}\mu\text{m}$ particles were less than the rates for $7.0\text{-}\mu\text{m}$ particles. For equal numbers of saltating particles, the resuspension rate increased with saltating particle size.

Velocity effects for $7.0\text{-}\mu\text{m}$ bed particles are shown in Fig. 1. Equal masses of saltation particles resulted in highest total resuspension rates for the $100\text{-}\mu\text{m}$ saltation runs. However, saltation with the $200\text{-}\mu\text{m}$ particles produced higher particle concentrations at 7 cm above the resuspension surface.

Vertical fluxes^{1,2} (vertical mass flow positive upward) ranged over three orders of magnitude for tests at a specific air velocity using equal numbers of saltating particles (Fig. 2). The change in vertical flux from no saltation to saltation with $200\text{-}\mu\text{m}$ particles was greater for $3.7\text{-}\mu\text{m}$ bed particles

Fig. 1. Particulate resuspension rates for equal masses of saltation particles.

Fig. 2. Vertical flux at 9.5 m/s centerline velocity for equal numbers of saltation particles.

than for 7.0- μm bed particles. Surprisingly, the maximum vertical flux occurred for particles greater than 10 μm from the 7.0- μm particle bed. The higher vertical flux with no saltation particles and 7.0- μm bed particles was not consistent with other results.

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Generation of Insoluble Aerosols for Inhalation Experiments

[In cooperation with the Mammalian Biology Group (H-4)]

Aerosol generation procedures have been developed to provide respirable particles similar to the radioactive particles used in the "hot particle" program.^{1,2} Small-animal inhalation experiments are being conducted with these aerosols to compare the biological effects on the lung resulting from inhalation vs injection and subsequent capture in the lung capillary network.

TABLE II

AEROSOL SIZE CHARACTERISTICS

Aerosol	AMAD (μm)	σ_g	Sizing Method
1	1.81	1.97	Impactor
1	1.96	2.50	Electron microscope
2	2.13	2.11	Impactor

The *in vivo* solubility of cobalt-57 encapsulated in heat-treated zirconium oxide particles³ has been tested by collecting the aerosol on a membrane filter, resuspending the particles in saline solution by ultrasonic agitation, and then injecting the solution into the leg muscle of hamsters. The hamsters were whole-body counted to determine the clearance rate and finally sacrificed to determine the distribution. Essentially all of the cobalt-57 remained at the injection site, indicating that the aerosol generation system provides particles of the desired characteristics (i.e., radionuclide encapsulated in zirconium oxide particles).

The size characteristics of the inhalation aerosols are given in Table II. The size of both aerosols was determined by cascade impactor and verified by electron microscope measurements of one aerosol. The agreement between the two methods indicates that the activity is homogeneously distributed in the particles. The particle size produced will have a significant respirable fraction.

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DETERMINATION OF PLUTONIUM IN MAN

[J. F. McInroy, H. A. Boyd, M. W. Stewart, M.D.
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Lung Distributions of Plutonium in Occupationally Exposed Individuals

Little is known concerning the actual distribution of plutonium within the human lung as a function of time after exposure. It is known that particle size, solubility of the plutonium compound, mode of exposure, duration of exposure, etc., all influence the distribution and retention parameters in each incident.

The lungs from two occupationally exposed individuals were inflated with dry nitrogen to approximately the size and shape the lung would occupy in the thoracic cavity. The inflated lungs were frozen, placed in an insulated container with Dry Ice, and counted with the LASL chest counter to compare the results with the last *in vivo* lung count before death. These results are shown in Table I.

The left superior lobe was removed and cut into transverse sections as shown in Fig. 1a. The sections from case 7-138, a former worker with 26 yr of potential exposure, were divided into a pleural-subpleural tissue fraction and the rest identified as parenchymal tissue (Fig. 1b). Each fraction was

TABLE I

LUNG COUNTER MEASUREMENTS OF LUNG BURDEN
OF PLUTONIUM-239 AND AMERICIUM-241

	Estimated Lung Burden	
	²³⁹ Pu (nCi)	²⁴¹ Am (nCi)
<u>Case Number 7-138</u>		
February 1972 (<i>in vivo</i>)	< MDA ^a	3.6 ± 0.7 ^b
August 1973 (<i>in vitro</i>)	19 ± 4	2.6 ± 0.5
<u>Case Number 18-002^c</u>		
November 1974 (<i>in vivo</i>)	< MDA	0.15 ± 0.03
December 1974 (<i>in vitro</i>)	< MDA	0.25 ± 0.05

^a Minimum Detectable Activity (MDA) for plutonium-239 and americium-241 are approximately 9 and 0.2 nCi, respectively.

^b Stated errors are ± one standard deviation.

^c Left chest and lung measurements only.

LEFT LUNG - SUPERIOR LOBE

Fig. 1. Schematic representation of the lung, indicating the transverse sectioning of the lobe and the division of each section into pleural and parenchymal subsections.

weighed and analyzed for plutonium using the standard analytical procedure for tissue analysis.

The ratio of plutonium concentration in the pleural-subpleural fraction to the parenchyma ranged from 10 to 37 in four sections (Table II). Unfortunately, analytical results for the subsequent sections between this region and middle of the lobe did not provide an accurate estimate of the chemical recovery; consequently, there are uncertainties as to their absolute values. They do indicate a decreased concentration in the pleural sections toward the mid-portion of the lung. These results are similar to those of Nelson¹ who reported concentrations 59 times greater in the pleural and subpleural tissues than in the parenchyma in a former worker with 25 yr of potential exposure.

The second case (18-002) had been exposed approximately one month before death in an accident. The inflated left superior lobe was sectioned into

TABLE II

DISTRIBUTION OF PLUTONIUM IN THE LUNG
OF A LONG-TERM EXPOSURE CASE (7-138)

Distance from Apex (mm)	Concentration		Ratio (pleura to parenchyma)
	Pleura (pCi/kg)	Parenchyma (pCi/kg)	
16	3.2 × 10 ⁵	1.5 × 10 ⁴	21
20	1.4 × 10 ⁵	7.1 × 10 ³	20
24	8.8 × 10 ⁴	8.5 × 10 ³	10
28	2.3 × 10 ⁵	6.2 × 10 ³	37

transverse sections approximately 3 to 4 mm thick. Each section was subdivided into the pleural-subpleural tissue (~2 to 5 mm wide) and either two or three parenchymal sections (1 to 2 cm wide) (see Fig. 1c). Each section was chemically analyzed for plutonium, and the results are shown in Fig. 2. The distribution of plutonium in the various lung sections of this recent exposure case shows relatively little variation in concentration between the parenchymal and pleural regions. However, there was an apparent increase in plutonium concentration in both regions as one proceeds inferiorly from the apex of the lung. Near the middle of the lobe, the parenchymal sections were divided into three sub-sections: one that contained the outer 1 to 2 cm of lung, an intermediate portion 2 to 4 cm deep, and an inner section containing the major bronchi (Fig. 1c). Again, there was little difference between the outer two regions of parenchyma, but the concentration in the region containing the bronchi appeared lower.

The data from these two occupationally exposed cases suggest that the plutonium is fairly uniformly deposited throughout the lung at reasonably short times following exposure. As time passes, there seems to be concentration in some subpleural regions either by migration toward the pleura or preferential elimination from the parenchyma, or a

combination of both of these mechanisms. More data are needed to confirm these observations.

Sections from the lung of the previously mentioned long-term exposure case (7-138) were fixed in 10% formalin and prepared for histological examination by the local pathologist. Pathological examination of the lung sections revealed essentially normal histological patterns for the age of the deceased. There were focal nodules of pleural and interstitial fibrosis and anthracosis without evidence of active granulomatous disease or active fibroplasia. The pulmonary parenchymal tissues peripheral to these areas showed evidence of possible slight emphysematous change. There were no microscopic changes that were inconsistent with the normal aging processes.

Alternate slides of the lung tissues were prepared for autoradiography. After a 5-wk exposure, the autoradiographs were developed and examined for alpha tracks. Assuming the tracks are from $^{239}\text{PuO}_2$, estimates of the particle sizes were made using previously reported techniques.² The mass median diameter of the lung particles was 0.16 μm , with a geometric standard deviation of 1.4, and all the particles observed contained less than 0.01 pCi of plutonium. This can be compared with the similar analysis of particle sizes in the tracheobronchial lymph nodes (TBLN) from this same case.³ The mass median diameter in the TBLN was 0.32 μm , with a geometric standard deviation of 1.5. The larger size observed in the lymph nodes may be the result of aggregation of particles by the macrophages in translocation of the particles to the lymph nodes. This phenomenon has been reported in dogs by Dagle *et al.*⁴ The technique used in our procedure does not identify whether the plutonium star was produced by a single particle or by an aggregation of particles.

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Plutonium in the Tissues of the General Population

Data are being accumulated to establish baseline concentrations of environmental plutonium in the non-occupationally exposed general population.

TABLE III

NUMBER OF AUTOPSY CASES RECEIVED
FROM THROUGHOUT THE UNITED STATES

Area	Number of Cases Received	
	CY 1975	1959-1975
Colorado	61	307
Georgia-South Carolina	40	100
Illinois	30	41
Pennsylvania	108	221
New York	0	36
New Mexico (including LASL)	16	376
Tennessee	a	a
Total	255	1081

^a Approximately 500 formalin-fixed autopsy cases were received, consisting of small pieces of organs. Attempts will be made to pool the specimens.

This information is essential for assessment of risk parameters associated with the growth of the nuclear industry. Tissues from the general population of seven geographic locations are being collected and analyzed. The number of autopsy cases received to date is shown in Table III, and the median concentration of plutonium in all tissues analyzed is shown in Table IV. The highest concentrations of plutonium in this population are in the pulmonary lymph nodes, liver, lung, and bone. The median concentration in kidney and gonadal tissue was below the minimum detectable level of 0.01 pCi/sample analyzed. The data from the Los Alamos cases are noticeably higher than from the other localities. This may be due to a large number of cases from older residents who lived in Los Alamos at a time when possible release of radioisotopes to the environment was not controlled to the level required by present regulations and provided by current engineering controls. Therefore, these cases may not be typical of the true general population. The lower concentrations observed in the first 40 Pennsylvania cases analyzed may be a reflection of the decreasing fallout levels of plutonium, since all these cases represent deaths that occurred during 1974 and are the most recent cases analyzed. The other areas

TABLE IV

FIFTIETH PERCENTILE DISTRIBUTION OF PLUTONIUM IN HUMAN TISSUE FOR THE NON-OCCUPATIONALLY EXPOSED (1959-1975)^a

Location	Lung	Liver	Lymph Nodes	Kidney	Vertebra	Gonad
Los Alamos	0.60 (66) ^b	0.56 (60)	1.51 (62)	0.12 (64)	0.22 (41)	c
New Mexico-United States ^d	0.36 (100)	0.46 (86)	1.07 (93)	< MRL ^e (91)	0.18 (66)	< MRL (9)
Colorado	0.25 (111)	0.77 (90)	0.56 (84)	< MRL (76)	0.19 (45) 0.39 (73) ^g	< MRL (61)
New York	0.30 (36)	0.62 (32)	f	f	0.37 (32)	0.34 (33)
Georgia-South Carolina	0.25 (20)	0.53 (14)	c	< MRL (12)	< MRL (13)	c
Pennsylvania	0.13 (40)	0.54 (39)	< MRL (21)	< MRL (39)	< MRL (18) ^g	< MRL (18)
All Populations	0.27 (373)	0.57 (321)	1.35 (260)	< MRL (281)	0.23 (197)	< MRL (221)

^a In pCi/kg.

^b Number of samples analyzed.

^c Insufficient number analyzed for statistical significance.

^d Cases that died and/or were autopsied in Los Alamos but were not residents of Los Alamos.

^e Minimum reporting levels.

^f Samples not requested and/or not received.

^g Rib specimens (not included in "All Populations" data).

are represented by cases that have died over the previous 10 to 15 yr, which include periods of higher fallout from atmospheric weapons testing. This hypothesis requires careful statistical study.

Statistical evaluation of the differences in median concentration values is currently in progress, but only the Colorado data have been examined in detail at this time.¹ Preliminary results indicate that the Colorado lung, tracheobronchial lymph node (TBLN), kidney, and gonadal tissue median concentrations are statistically lower than the non-Colorado concentrations. The Colorado median liver concentration was statistically higher than the non-Colorado data, and there was no significant difference in the vertebral concentrations. Comparison of the ninetieth percentile concentrations, using one-sided non-parametric tolerance intervals and making no assumptions except that the distributions be continuous, demonstrated that 90% of the population measured had tissue concentrations less than the figure given in Table V at the 90% level of confidence. The data include all replicate analyses. At this upper end of the concentration range, the Colorado lung, lymph node, and vertebral concentrations are lower and the liver and kidney concentrations are higher than the non-Colorado analyses. The gonadal concentrations are about the same. The significant differences observed at the ninetieth percentile (Table V), compared with the test of means (Table IV), are the relative concentrations in the kidney (Colorado > non-Colorado), vertebra (Colorado < non-Colorado), and gonad (Colorado $\approx$ non-Colorado). No differences were found to be significant between male and female tissue concentrations of plutonium, and no differences were evident between communities in Colorado, with

TABLE V

NINETIETH PERCENTILE CONCENTRATIONS
OF PLUTONIUM IN HUMAN AUTOPSY TISSUES

<u>Tissue</u>	<u>Non-Colorado (pCi/kg)</u>	<u>Colorado (pCi/kg)</u>
Liver	1.9	3.7
Lung	2.9	1.3
Lymph Node	82.4	78.4
Kidney	1.9	4.9
Gonad	37.0	36.9
Vertebra	10.6	5.7

the exception of the kidney concentrations. No reasons for this discrepancy are known at this time.

Evaluation of the data from other localities is continuing. The effect of analytical parameters such as aliquot size, presence of diseased tissue, and small tissue or organ sizes on the extrapolated tissue concentrations is being investigated.

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Health of LASL Plutonium Workers

[G. L. Voelz, M.D., and L. H. Hempelmann, M.D.]

The on-going study of 26 Manhattan Project workers exposed heavily to plutonium in 1945-1946 continues to attract considerable attention due to the unique value of their exposure history in evaluating possible health effects. The value of these observations on only a few individuals pointed to the need to extend the study to more subjects; this has been the main effort during 1975. Table VI shows the results of our efforts as of September 1975 to locate the 260 persons who have had the

TABLE VI

SUMMARY OF LASL PLUTONIUM WORKERS STUDY

	<u>Located</u>	<u>Not Located</u>	<u>Total</u>
Current Employees	90	0	90
Former Employees	115	33	148
Deceased	<u>22</u>	<u>0</u>	<u>22</u>
	227	33	260
Percentage	87%	13%	

TABLE VII

AGE DISTRIBUTION OF LASL PLUTONIUM WORKERS STUDY

<u>Birth Year</u>	<u>Living</u>	<u>Deceased</u>
< 1900	3	6
1900-1904	3	2
1905-1909	12	1
1910-1914	20	2
1915-1919	60	3
1920-1924	84	6
1925-1929	20	2
1930-1934	1	0
1935-1939	2	0

TABLE VIII

BODY BURDEN ESTIMATES

nCi	Located	Years of Exposure	Unlocated	Years of Exposure	Deceased	Years of Exposure
> 10	17		9		1	
10-19	117		19		11	
20-39	41	19 ^a	5	26 ^a	9	12 ^a
40-79	15	12 ^b			1	16 ^b
80-119	5	19 ^b				
120-159	5	29 ^b				
160-199	2	25 ^b				
200-239	2	31 ^b				
240-279	1	31 ^b				

^a Average years of exposure since reaching 20 nCi estimated.

^b Average years of exposure since reaching 40 nCi estimated.

highest plutonium exposures at Los Alamos. Efforts to locate the 33 missing persons are continuing.

The age distribution of the 227 located persons is shown in Table VII. It is noted that 89% of the group is over 50 yr of age. The distribution of the estimated plutonium body burdens by the urinary excretion method used at LASL (PUQFUA code) is shown in Table VIII. This table shows the number of persons located, unlocated, or deceased by body burden categories and the years of internal exposure since reaching either 20 or 40 nCi. All 31 persons with exposures above 40 nCi and 81 out of 86 (94%) of those above 20 nCi have been located. The U. S. Transuranium Registry has identified approximately 160 workers who have exceeded 20 nCi exposure (50% of the maximum permissible body burden in the history of all ERDA facilities). Our group then represents approximately half of all the United States human experience with plutonium exposures above these levels.

The located individuals have had either a physical examination (mostly current employees) or responded to a mail questionnaire on their health status. This gives us some health information on at least the major problems known to these persons, although the diagnoses have not been verified yet through their physicians or hospital records.

The presence of neoplasms is a major category of interest as a potential long-term effect of plutonium. There are five cases reported in our located

living persons: melanoma, lymphoma, carcinoma of the colon, stomach "cancer," and epidermoid carcinoma of the lip. The causes of death within the group are listed in Table IX. No conclusions can be drawn from such small numbers, although the list does not seem unusual or remarkable. It is probably fortuitous that the leading neoplasm in males, carcinoma of the lung, is not yet present in our series. This tumor is also of interest in relationship to inhalation exposure to plutonium. All cancers, including the five cases in the living persons, have been in persons in exposure groups less than 20 nCi body burden.

TABLE IX

CAUSES OF DEATH IN LASL PLUTONIUM WORKERS STUDY

Accidents	5
Myocardial Infarct	3
Neoplasms	3
Carcinoma of colon	
Carcinoma of urinary bladder	
Melanoma	
Suicide	2
Pneumonia	1
Post-Operative: Coarctation of Aorta	1
Cirrhosis	1
Unknown ^a	6
Total	22

^a Death certificates have not been received yet on these cases, and no other information is available.

Analysis and Assessment

[J. W. Healy, R. L. Thomas, and W. J. Wenzel]

Plutonium and the transuranics are controversial elements because, at least partially, their effects on health of people and their environmental transport are uncertain. While much information is available in the literature, it is usually uncoordinated and not in a form to provide answers, particularly to broad questions. This project is intended to provide an in-depth study of the data, with the main goals those of understanding the scientific basis for any conclusion and identification of areas where further research is needed.

To date, much of the effort has been devoted to analysis of questions raised by others, such as the

hot particle problem or the selective irradiation of the bronchi, and preparation of critical reviews. Considerable time and study were devoted to plutonium hazards for the LMFBR environmental impact statement, including a review paper on uptake in the gonads. A study of the uptake in plants of americium and other transplutoniums is in progress, along with an updating of material on methods of determining the costs of reactor accidents used in the Rasmussen report. The Proceedings of the Second Life Sciences Symposium on "Plutonium -- Health Implications for Man" appeared in the October issue of Health Physics.

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INTRODUCTION

Environmental research activities during 1975 have expanded to include work on the environmental fate and effects of materials emitted from coal operations, uranium mill tailings rehabilitation, and effects of dry geothermal energy extraction. Work continued in programs focusing on the ecological behavior of plutonium and other transuranics in natural systems, studies in Alaska on the ecological effect of the oil pipeline construction and worldwide fallout radionuclide behavior in

arctic ecosystems, and terrain influence on meteorological transport.

These efforts may be characterized as field-oriented, with an interdisciplinary approach in both nuclear and non-nuclear areas. The results of these programmatic efforts are extremely valuable in assessing the interaction of man's activities with the biosphere. The summaries that follow will outline briefly current progress in the above-mentioned areas of investigation. Further detail is available from the publications list in Appendix A.

ENVIRONMENTAL STUDIES

Ecological Aspects of Uranium Tailings Rehabilitation

[C. L. Wienke and D. R. Dreesen]

The prime objective of this research program is to assess the ecological hazards posed by uranium mill tailings, while concurrently considering possible rehabilitation methods. The arid areas of the western United States are expected to experience a boom in uranium mining and milling. Past exploitation of this resource has already resulted in abandoned mill tailings piles at 21 uranium mill sites which contain approximately 25 000 000 tons of tailings. Limited studies on the ecological hazards posed by these tailings have been undertaken previously. Problems that are being addressed include the content of toxic and radioactive species within these piles, possible modes of transport of these elements out of the piles, and fate of these elements in the environs of the mill sites. After reliable data exist on the hazards the piles may produce, feasible alternatives for the amelioration of these hazards can be considered. These studies were begun in July 1975.

Initial efforts were to assimilate the available information from federal, state, and private agencies to expedite the studies and to avoid duplication of effort. Liaison was established with ERDA DOS personnel and contractors to integrate the engineering and ecological aspects of mill tailings rehabilitation that have different funding sources. Initial contacts have been made with responsible agencies such as the New Mexico Environmental Improvement Agency, U. S. Environmental Protection Agency, and Navajo Nation.

Initial study efforts include an inventory of uranium radioactive decay daughters in the tailings, as well as nutrient and potentially toxic trace elements such as selenium, mercury, lead, arsenic, vanadium, and molybdenum. Delineation of toxicological factors which may affect tailings revegetation will be of considerable importance. With this initial inventory, it will be possible to narrow research tasks for subsequent intensive investigation. These investigations are defining transport of ecologically significant constituents from the pile. Particulate resuspension, gaseous diffusion,

leaching, and surface runoff from the tailings piles are transport mechanisms being studied. The transport mechanism(s) determined to be the most ecologically hazardous will become the basis for judging the rehabilitation options for their feasibility and ecological desirability.

The significance of this study lies in the development of basic ecological information applicable to tailings rehabilitation technology. The arid western United States is an area where a relatively small amount of water pollution can have magnified consequences because of relative water scarcity. This area is characterized by ecosystems which are more visibly upset by energy extraction activities than are other regions.

Ecological Fate and Effect of Trace Elements from Coal Utilization in the Semi-Arid West

[L. E. Wangen, C. L. Wienke, D. R. Dreesen, B. A. Perkins, and M. L. Marple]

The long-range goal of this research, begun in FY 1976, is to determine the ecological fate of trace elements resulting from the extraction and utilization of coal in semi-arid regions of the western United States. This type of information is one of the prerequisites to the mitigation of possible adverse environmental effects resulting from extensive energy development in this region.

The extraction and combustion of coal change the physical and chemical properties of the trace species originally present in the coal matrix. Some of these processes are known to convert various toxic materials from a relatively inert, immobile state to physical and/or chemical forms that are more available for uptake or movement in the environment. For example, the high-temperature combustion process volatilizes many species, thereby enabling their escape to the atmosphere and subsequent dispersal over large geographic regions. A less studied phenomenon is the potential mobilization of trace contaminants from coal ash after disposal such as that resulting from wind and water erosion. For example, current and planned utilization of strip-mined coal for conventional power production by combustion in northwestern New Mexico is on the order of 10 000 000 tons/yr. This coal has an unusually high ash content of about 20%. Thus, on an annual basis, 2 000 000 tons of coal ash must be disposed of in some way. The possibility of

water contamination by leaching and surface runoff of trace contaminants from strip-mined areas and ash disposal sites is particularly noteworthy in an area where the limiting natural resource is the quality and quantity of water. Additionally, transport, dispersion, and deposition of airborne plumes in this region are not well characterized due to insufficient meteorological data and a lack of information on terrain effects. This makes it extremely difficult to assess, with any accuracy, the effects of fumigation and trace element deposition.

The Four Corners area of New Mexico was chosen as the study locale for this research, the principal reasons being its proximity to Los Alamos; the opportunity to coordinate and cooperate with other research programs being carried out in the same area; very extensive coal mining, coal combustion, and proposed coal gasification operations in the area; and indications of cooperation from the Navajo Nation and the public utility company operating the Four Corners power plant (i.e., the Arizona Public Service Company).

A multidisciplinary approach encompassing the atmospheric, biological, and earth sciences is being used to study the movement of trace elements. The influence of terrain on plume transport and dispersal and particle deposition is being studied with the intent of developing a model capable of predicting deposition patterns in the Four Corners region. Wind resuspension and water leaching of trace elements from the ash disposal ponds is to be studied, and air particulate samples, collected using particle sizing samplers, will be analyzed for selected trace elements. Laboratory studies on the water leaching of trace elements from ash as a function of pH and various other chemical parameters are being implemented. The use of indigenous soil microbial populations and lichens as possible trace element indicator organisms is being investigated.

The available literature on programmatic and toxic element research was reviewed and evaluated, after which the above research tasks were defined. Judgments were made as to which ecosystem components might be most noticeably affected by the trace element output of the Four Corners power plant. Priorities of approximately 25 trace elements were

established on the basis of toxicity, availability for uptake or mobilization, enrichment by combustion, and total output from the power plant. Trace elements presently under consideration include beryllium, boron, selenium, mercury, arsenic, and molybdenum. Contacts were established with other groups with commercial or scientific interest in energy extraction and utilization in the Four Corners area. Permission was obtained from the Navajo Nation to conduct initial studies, and tentative cooperation has been promised by representatives of the Navajo mine (Utah International, Inc.) and the Four Corners power plant (Arizona Public Service Company).

Efforts were begun to acquire meteorological and air quality monitoring data from existing sources. Simple gaussian plume modeling of particulate deposition and development of a wind field model to account for terrain effects using small perturbation theory have begun. Samples of soils, in-plant ash, scrubber slurries, dried ash from disposal ponds, seepage and decantation from ash ponds, inlet and outlet water from Morgan Lake, and other sources of interest were obtained during late September. A technique for in-field filtering of liquids was tested and found satisfactory. This sample survey is intended to assist further in the prioritization of trace elements and potential problem areas. The samples are being analyzed for selected trace elements by emission spectrographic, neutron activation, and atomic absorption techniques. An aerial survey of the power plant area was made, during which dominant terrain and other topographic features were identified and reference photographs made (Fig. 1). Preliminary soil microbiological

Fig. 1. The Four Corners coal-fired power plant environmental study area in New Mexico.

experiments aimed at determining the sensitivity of soil microbes to various trace elements have been performed. Initial results of these studies, based on the hypothesis that indigenous soil bacteria not adapted to metals are more sensitive to an additional metals burden than those bacteria which had adapted to these same metals, indicate the desirability of continuing this effort.

Radioecological Investigations at Los Alamos and Trinity Site

[T. E. Hakanson and J. W. Nyhan]

Despite the importance of plutonium in nuclear power production, the distribution and long-term behavior of this element and other radionuclides are not well characterized in various ecosystems. Long- and short-term studies were initiated on the environmental distribution, transport, and biological availability of plutonium, americium, and other radionuclides originating from treated liquid industrial wastes released to three canyons at Los Alamos and from nuclear weapons debris in the arid Trinity Site environs. The studies involve radionuclides which have weathered for as long as 32 yr in both aquatic and terrestrial ecosystems. Radionuclide studies were coupled with quantitative surveys of the soils and biota to provide a basis for compartmental analysis of plutonium and other radioactive and stable elements in the study ecosystems.

Data currently available show that soils and alluvium contained the largest portion of plutonium added to the Los Alamos and Trinity environs. However, the horizontal, vertical, and fractional distribution of soil plutonium in the alluvial soils at Los Alamos differed from the terrestrial soils at Trinity. Plutonium concentrations in Los Alamos alluvium steadily decreased with distance from the waste outfall and did not exhibit consistent patterns with alluvial depth. The horizontal and vertical distribution of plutonium in the canyons at Los Alamos is partially governed by storm runoff processes. In contrast, the horizontal distribution of plutonium in Trinity soils was largely determined by the original post-detonation deposition pattern of plutonium; levels were correlated with the penetration depths of rainwater into soil profiles. The smallest amounts of plutonium were consistently found in the $< 53\text{-}\mu\text{m}$ -size fraction of

the coarse-textured Los Alamos alluvial soils, whereas the Trinity soils contained elevated amounts of plutonium in coarser size fractions ($> 105\text{ }\mu\text{m}$) near Ground Zero and relatively larger amounts in fine fractions at increasing distances from Ground Zero. Studies were recently initiated to characterize wind attendant soil resuspension as a plutonium transport vector at Trinity.

Since runoff from summer rainstorms was implicated as a major transport mechanism at Los Alamos, repeated field gamma-ray measurements and sampling of runoff and alluvium suggested a seasonal pattern of radionuclide distributions; buildup occurred from fall until spring, followed by extensive losses during runoff events from July through September. Since some of the intermittent streams receiving treated radioactive wastes occasionally flow to the Rio Grande River, sediment and fish samples were collected along the Rio Grande at each of the major canyons draining the Los Alamos area; data currently available indicate non-detectable to worldwide fallout levels of radionuclides in river samples.

There is very little evidence to show that plutonium is concentrated in vegetation to levels exceeding those in the associated soils. Plant-soil plutonium activity ratios ranged from about 0.001 to 1.0 at Los Alamos and 0.0001 to 0.1 at Trinity Site and were consistently higher in lower growth forms such as the grasses, lichens, and mosses, suggesting that surface area relationships may be a factor in plant contamination. Similar cesium-137 to plutonium activity ratios in soils and associated vegetation are indicative of an external contamination on vegetation surfaces. Initial results of controlled experiments on root uptake of plutonium by bluegrass suggest that the physiological availability of this element to vegetation is extremely low in the canyons at Los Alamos.

Concentrations of plutonium in various tissues of herbivorous and carnivorous animals from the Los Alamos and Trinity study areas indicate a low physiological availability of this element. Generally higher concentrations of plutonium in lung and pelt tissues suggest that resuspended soil is being inhaled and deposited on external surfaces. The physiological availability of plutonium and other

radionuclides in the liquid effluent to native deer mice was negligible, as determined by controlled feeding experiments.

Field experiments in one of the canyons containing trace levels of radioactivity indicated that gamma radiation exposures to native rodent populations implanted with thermoluminescent dosimeters are species-dependent, with less mobile species accumulating larger total exposure through equivalent time intervals. In addition, all species accumulated less than 30% of the gamma exposures measured with field survey instruments in the study area. The time rodents spend underground in dens possibly accounts for the lower exposures in these burrowing mammals.

Since soils contain most of the plutonium present in the study areas, the long-term disposition of this element will be governed by the physical and biochemical processes operative in soil formation and transport. Differences between plutonium distribution with particle size and depth in the study areas reflect some of these physical and biochemical dissimilarities and have some important implications as to potential transport mechanisms within the soils and biotic components of the ecosystem. Observations on these processes now under way at Los Alamos and Trinity require study over a long period.

Consistently higher concentrations of plutonium in grasses from both study areas likely reflect higher entrapment efficiencies for those species due to greater surface areas. This would indicate that the relative proportion of grass in the diet of wild and domestic herbivores has an important bearing on the quantity of plutonium ingested.

Ecological Studies of Non-Nuclear Materials in the Los Alamos Environs

[T. E. Hakonson and F. R. Miera, Jr.]

The Los Alamos Scientific Laboratory has several on-site areas which contain various non-radioactive elements typically found as effluents or by-products of geothermal and coal field development and their associated energy production facilities. Studies on natural uranium, depleted uranium, and mercury were initiated recently in a conventional explosives test area and in three canyon liquid effluent receiving areas where radionuclide studies are under way to gather data on the

ecological distribution and transport pathways of these actinides.

Several thousand kilograms of natural uranium and depleted uranium were dispersed to the environment in the explosives test area. Additionally, kilogram quantities of mercury, cadmium, lead, nitrate, and other metals have been utilized in the explosives test area and in the effluent receiving canyons. Many of these elements were released within the study areas nearly 32 yr ago and have been exposed to aging-weathering processes for that period, thereby providing an attractive study opportunity.

Studies of uranium-depleted uranium in major explosives testing areas were conducted in conjunction with comparative studies of vegetation and animal communities to lay the foundation for a compartmental analysis of environmental uranium-depleted uranium. Soils contained most of the residual uranium-depleted uranium in the study areas, with vegetation and small mammals containing relatively minor amounts. Ratios of plant/soil uranium-depleted uranium concentrations varied from 0.08 during November to 0.05 during June, or within the range reported from other studies of plants in high natural uranium areas.

Mercury studies were begun recently in the canyon waste disposal areas where studies on plutonium and other radionuclides have been under way since 1972. Procedures for mercury analyses in soils and biota are currently under development. A portion of the samples, routinely collected from the canyons, will be subjected to mercury determinations to provide a data base on the distribution of this element in the study ecosystems. Comparisons with radionuclide concentration data will be made to identify similarities in distributions, providing insight to the mechanisms operative in waste redistribution.

Environmental Aspects of Energy Extraction from Dry Hot Rock Geothermal Resources

[A. K. Stoker, D. R. Dreesen, F. R. Miera, Jr., and W. D. Purtymun]

The overall objective for this project is to develop a basic understanding of the environmental consequences of producing energy from dry hot rock geothermal resources. The focus is a case study of environmental effects of the demonstration

experiment being conducted at the Fenton Hill site (Fig. 2) in the Jemez Mountains of northern New Mexico by the LASL Energy Division. The technical experiment expects to demonstrate the possibility of extracting heat from dry hot rock ($\sim 200^{\circ}\text{C}$) by circulating water through artificially induced fractures connecting two bore holes drilled into granitic rock to depths of about 3000 m (10 000 ft). The environmental study will develop the information for documenting and assessing the actual, potential, and perceived environmental problems associated with operations at the experimental site which may eventually include a pilot electric generating station.

Investigations in the areas of ecology, hydrology, and atmospheric science constitute the core of the formal program initiated in FY 1976. Some work in hydrology and ecology was started earlier to coordinate with critical activities at the experimental site. A detailed evaluation of the complex regional surface and ground water hydrology, including review of relevant literature and field studies, provided the basis for establishing a water quality surveillance network. Sixteen locations representing surface water, springs, and wells are sampled on a regular schedule which started in 1974 to provide base-line characterization of

physical and chemical water quality in the vicinity of the demonstration site. The surface water quality in the drainage area of the Jemez River is determined largely by the quality of in-flow from thermal and mineral springs. Monitoring is being continued and extended to include additional analyses for minor chemical constituents of potential importance. Additional site-specific hydrologic studies are under way to evaluate the impacts on runoff, erosion, and seepage due to the physical disturbances of site preparation and drilling operations.

Preliminary ecological inventories of vegetation and small mammals in the vicinity of the demonstration site have been conducted. The site is located at the edge of an area burned by a major forest fire in 1971 and encompasses two basic plant communities. One is a ponderosa pine- (*Pinus ponderosa*) Douglas fir (*Pseudotsuga taxifolia*) community, and the other is a fire seral community dominated by aspen (*Populus tremuloides*), various forbs, and grasses. Preliminary results indicated a larger small-mammal population in the fire seral community, and the deer mouse (*Peromyscus maniculatus*) was the dominant small mammal observed in both plant communities. More extensive ecologic studies are under way to evaluate such things as primary productivity and cycling of nutrients or toxic substances in the ecosystems.

Atmospheric studies will include climatological monitoring in support of the ecological and hydrological studies. Base-line air quality sampling will be conducted for constituents of potential importance.

All components of the environmental study are being coordinated closely with the technical experiment in order to anticipate potential environmental effects such as those that may result if disposal of liquid effluents becomes necessary. Seismic monitoring data will be available for interpretation from an environmental hazard point of view.

This study is of particular significance because it is the vehicle for documenting the environmental implications of a particular technology for exploiting geothermal resources that is potentially one of the least polluting. An understanding of the environmental effects of this demonstration will be essential in evaluating potential commercial-scale development.

Fig. 2. The Fenton Hill site of the LASL dry geothermal source demonstration project is located in the Jemez Mountains of northern New Mexico about 32 km (20 air miles) west of Los Alamos. Drilling of the second hole is under way (center), while experiments are conducted in the first hole (left, center).

Radiation Ecology Studies of Northern Alaska

[W. C. Hanson]

Radiation ecology studies of northern Alaska have been conducted continuously since 1959 to evaluate the transport and fate of worldwide fallout radionuclides in the relatively simple ecosystems that effectively transmit materials to man and other carnivores of that region. These data now provide an unexcelled base upon which to estimate rather precisely for the first time the feeding rates of free-ranging populations of caribou and their ecological dependents; to relate their intake of various stable chemical analogs to the mineral pools of major importance in arctic tundra environments; to monitor health effects that may or may not be attributed to or correlated with atmospheric radioactivity and fallout; and to measure socio-economic impacts of changing life styles in northern Eskimos that result from resource developments in their environment.

The study initially surveyed all 13 of the major villages of northern Alaskan Eskimos and Indians that have ancestrally derived their livelihood from subsistence hunting and fishing over an area of about 150 000 square miles. Whole-body counting was conducted initially with a semi-portable shadow-shield counter constructed of 4-1/2 tons of lead bricks and associated electronics gear. About 4000 interviews were concomitantly obtained to relate food sources to the elevated fallout cesium-137 body burdens that were observed routinely. These data showed that the cesium-137 body burdens exceeded by two orders of magnitude those of other peoples of the United States and were directly correlated with the consumption rates of caribou/reindeer. Important differences were found in radionuclide concentrations from various environments, and the atmosphere-to-lichen-to-caribou-to-man food web was recognized early as the major source of both worldwide and natural fallout in the native residents of northern Alaska.

Following the general survey and ranking of various food-gathering cultures on the basis of transmissivity of fallout radionuclides, intensive studies were begun at Anaktuvuk Pass in 1962 and have continued to the present time. Cesium-137 concentrations in the three trophic levels at that location are shown in Fig. 3 and demonstrate the

Fig. 3. Cesium concentration in lichens, caribou flesh, and Eskimos at Anaktuvuk Pass, Alaska, during the period 1962-1975.

significant cycles that resulted from the migration of caribou between summer and winter ranges and the seasonal harvests by the Eskimos of those animals. These cycles were strongly influenced by the physiological parameters of nutrient content, plant growth, and seasonal temperature. Several of these mechanisms were investigated by controlled field studies in order to define more precisely the transfer coefficients and rate constants that were incorporated into a simulation model of the lichen-caribou-Eskimo food web. That model is now being refined during a relatively stable period of low fallout rates and will allow natural cycling phenomena to be observed that were previously masked by atmospheric nuclear tests. Such cycling effectively reduced by one-half the radiation exposure from cesium-137 body burdens in adult Anaktuvuk Pass Eskimos. The average annual exposures during the maximum period of 1964-1966 were about 140 mrad and are now about 50 to 70 mrad/yr. Reasonable skeletal dose rates from strontium-90 were estimated by a mathematical model to be 100 to 130 mrad/yr.

The substantial library of samples collected during the study are now being processed for transuranium elements such as plutonium-238, plutonium-239, plutonium-240 and for natural radionuclides such as lead-210 and polonium-210 that are similarly transferred through the arctic food webs. These data will be used to define further the cycling characteristics of heavy metals in arctic ecosystems

and to evaluate their contribution to the total radiation exposure of the northern Eskimos. Medical X-ray records of several of the people were summarized to evaluate that source of exposure as a contributor of radiation exposure following a study of chromosomal aberrations in the Anaktuvuk Pass people that indicated the presence of ring and dicentric anomalies. The total radiation history of the participants is being reconstructed in cooperation with the Battelle-Pacific Northwest Laboratory.

Cultural observations that were incidentally recorded during the study have now proved invaluable as the only complete history of the Anaktuvuk Pass Eskimos during a critical period of transition in cultural change. By combining these data with cesium-137 measurements in the people, their caribou food base, and other carnivores, it is possible to make an integrated assessment of social processes that are strongly influenced by current Alaskan North Slope oil exploration, extraction, and transport.

Ecological Investigations of Alaskan North Slope Oil Development

[W. C. Hanson and L. E. Eberhardt]

The objective of this study is to evaluate the ecological consequences of the construction and operation of the Trans-Alaska pipeline by means of selecting indicator organisms from tundra environments in the vicinity of arctic land disturbance for exploration, extraction, and transport of Alaskan North Slope petroleum.

Arctic (Fig. 4) and red fox populations were selected for study because (1) they are an important and relatively abundant carnivore of the region traversed by the pipeline and are closely dependent upon the small mammals and birds that may also be affected by oil development activities; (2) there is widespread but unsubstantiated opinion that red foxes are displacing the more valuable arctic foxes in certain areas of the Arctic Slope, although the two species tend to be associated with different types of habitat and alteration of the habitat by pipeline activities may have substantial effects upon species relationships; and (3) foxes have important relationships with human activities from the standpoints of their being a valuable fur resource, their destruction of equipment related to petroleum exploration and development, and their

Fig. 4. An adult arctic fox in winter pelage photographed near a Trans-Alaska pipeline construction camp during March 1975.

function as a reservoir for rabies. An important consideration for the future is the anticipated increase in fox-human contacts when the pipeline haul road, by present laws, becomes an Alaskan state highway, once oil begins to flow at the Valdez terminal.

Responsibility for the study is divided into two broad categories: (1) the basic biology of arctic and red foxes along the Arctic Slope portion of the pipeline route and (2) determination and monitoring of fox population densities and movements along the pipeline route and in Prudhoe Bay. In addition, prey populations of lemmings and shorebirds are mapped and counted to determine interactions of the foxes and their alternative food resources. A data base of such measurements that extends back to 1971 provides an extension of vital information.

During the past year, a total of 26 arctic fox dens were located in the Prudhoe Bay area, of which 11 were actively used by 9 fox families. An additional 30 arctic and red fox dens were noted along a 40-mile segment of the Trans-Alaska pipeline route in the Franklin Bluffs-Happy Valley region. Live-trapping operations in these areas and in a few pipeline construction camps during winter months

resulted in the tagging of 42 foxes. One tag was returned from a Canadian Eskimo trapper who collected the animal a month later at a location 720 km northeast of the release site.

The 1975 summer investigations included a complete census of summer resident birds, their distribution in habitats, and nesting phenology. Banding was accomplished on 93 birds of 7 species and resulted in recapture of 5 adult birds that were initially banded in 1972 and 7 that were banded in 1973. One bird initially banded in 1972 was recaptured in both 1973 and 1975, providing valuable data for population analysis. Correspondence with other bird banders indicated that many of the birds encountered in this investigation are winter residents in South America and annually perform circuitous flights of about 10 000 km to and from their habitats.

Live-trapping and tagging of lemmings were also conducted. A low in the population cycle was observed and had important implications to arctic fox predation on tundra nesting birds, which represent an alternate food supply.

Biological studies on foxes resulted in a large amount of data on arctic and red fox ranges, denning habits, behavior, food habits, and other phenomena that are now being summarized. Additional studies were begun of moose and carnivore populations of the Colville River area that will be seriously affected by oil developments in the U. S. Naval Petroleum Reserve No. 4 and Prudhoe Bay areas.

Meteorological Transport Processes in Complex Terrain

[S. Barr, W. E. Clements, and D. A. Dahl]

The objective of the present work is to establish a sound physical basis for addressing problems of topographic influence on meteorological transport. It is clear from previous work that surface influences can affect wind, temperature, and precipitation fields in ways that are not obvious without detailed study. Further, it is also known that no single model will serve to answer all terrain problems. However, a fundamental understanding of the important physical processes can lead to improved assessments of terrain influence and greatly enhance the confidence in environmental analyses. The environmental aspects of virtually all of the energy development technologies outlined in the DBER

Balanced Program Plan have a dependence on meteorological fields which are driven or seriously altered by the topographic structure. Most power production facilities have emissions, often from tall stacks, which have environmental influences to be documented. The locations and extent of the deposited contaminants depend vitally on local flow and turbulence conditions. The increasing number of power facilities in areas of complex terrain underscores the need for understanding the role of topography in the local air flows and the atmospheric transport of pollutants.

There are a large number of practical diffusion models available for assessment purposes in the absence of significant terrain features, but only a few efforts have been initiated to take account of terrain in a systematic way. The existing models are unable to address the full range of problems to be encountered in siting and evaluation of energy production facilities.

A vital link in understanding complicated systems is the feedback between theory (or models) and experimental observation. Our research has been characterized by the essential concurrence of the two approaches. Also, in order to understand best the system under observation, we have chosen to begin with the simplest topographic structures available and to build systematically toward more complex structures. Our studies, then, reflect simple slope flows and canyon flows. We shall gradually increase the domain to canyon-mesa interactions, an ensemble of canyons and mesas present at Los Alamos, and finally to whole regions with their array of topographic features. The model development follows a similarly systematic line of approach from simple to sophisticated. This optimizes the opportunities for interpretation of results at each stage of development.

The work to date has focused on one canyon within which wind, temperature, turbulence, and tracer diffusion information has been collected for several seasons of the year. Figures 5 and 6 demonstrate some results from these surveys. In Fig. 5, a weak but steady down-canyon flow is established shortly after the cooling cycle starts and persists until sunrise. The turbulence is greater than would be expected over flat terrain for the same thermal stability, a phenomenon

Fig. 5. Los Alamos Canyon data: (A) canyon and slope flow component; (B) temperature; and (C) hourly range of wind direction.

Fig. 6. Schematic sketch of the persistent eddy in Los Alamos Canyon.

observed at a few other sites. Figure 6 shows the eddy which develops in the canyon under cross-canyon free stream flow. This is a frequent occurrence in this particular canyon and a major factor in determining the transport and diffusion of pollutants.

Model development has involved a growth from fundamental principles through a hierarchy of sophistication which maintains enough flexibility to incorporate phenomena of the type described above. Scale analysis of the governing hydrodynamic equations has provided a basis for comparison of several slope wind models. Thermal forcing is a key element to an understanding of terrain motions and has been studied.

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INTRODUCTION

The brief summaries which follow describe a selective aspect of each of the research programs which collectively comprise the Cellular and Molecular Biology Group. No attempt has been made to cover the interests of the principal investigators in a comprehensive manner. The general, overall focus of the group is to understand the functions of cultured mammalian cells in chemical terms. The rationale follows from the perception that the cell is a chemical entity and is appropriately understood in its own terms.

With the realization of the powerful synergism which can result from focusing multiple insights and technical expertise on problems, we have made a conscientious effort to develop fruitful collaborative efforts. Many of the summaries reflect such interactions. In addition to providing additional degrees of freedom to move in more scientific directions, one can detect a noticeable broadening in interest even among individuals not currently engaged in collaboration. Our most sustained multidisciplinary effort has concentrated on the mammalian cell cycle. This effort has brought together the combined efforts of cell biologists, biochemists, enzymologists, biophysicists, and engineers and has resulted in a greatly increased knowledge of the

biochemical events which occur at unique stages of the proliferative cycle. As we continue to foster such interdisciplinary research, we would hope to continue to cross barriers which have traditionally compartmentalized biology.

Our continuing examination of the ERDA mission as it relates to health effects leads us to conclude that the most extensive screening protocol that can presently be devised for carcinogenicity and mutagenicity, which have already received considerable thought and attention, would still leave significant questions unanswered. When one adds less well studied end points such as teratogenesis and central nervous system effect, it becomes obvious that we must make significant advances in our understanding of each of these defects and utilize the emerging general principles to divide toxic agents into mechanistic classes of manageable proportion and to devise simpler, more sensitive test systems to define their effects. No single approach will meet the demands of such a comprehensive effort, but we are convinced that the mammalian cell in culture is a useful test system for some agents and will help to provide some of the general principles needed to ensure a viable program which can respond to changing demands.

STUDIES WITH MODEL DNA POLYMERS

Physico-Chemical Measurement of the Effect of X Irradiation on Model DNAs

[F. N. Hayes, D. E. Hoard, D. L. Williams, E. L. Martinez, V. E. Mitchell, and E. C. Wilmoth]

It is generally conceded that the effects produced in living organisms by ionizing radiation (death, development of neoplastic disease, or production of mutated offspring) involve chemical modification of the genetic material. A detailed knowledge of what the chemical modifications are and how they are produced is necessary if we are to make proper assessment of the hazards associated with nuclear energy production or to design suitable remedial measures.

Except in certain viruses, the universal genetic material is deoxyribonucleic acid (DNA). Studies that have been conducted with isolated natural DNA indicate that both breakage of the molecules into shorter pieces and alteration or removal of constituent base moieties are the important types of damage produced by ionizing radiation. However, the base sequence in natural DNA is so complicated that further chemical characterization of damage by means of such studies is very difficult.

We have synthesized and extensively characterized a variety of model DNAs having known base sequences by a combination of organic-chemical and enzymatic procedures. The number of strand breaks produced after exposure to x irradiation can be determined from changes produced in the profile of the gel-permeation chromatograms of the materials. Thermodynamic analysis of the change in melting transition produced by ionizing radiation allows one to determine how much base damage has occurred.

Lowering of the temperature at the mid-point of the melting transition (T_m) of double-strand DNAs which is caused by both single-strand breaks and base damage has been established by appropriate experiments in which the model DNAs were modified chemically. The effect produced by a single-strand break varies with length of the strand, but in a strand containing 140 bases it is 3.3 or more times as great as that caused by damage of a single base. If double-strand molecules are formed from single strands, one of which has been x irradiated, the

Fig. 1. Depression in the mid-point of the melting transition (T_m) of a model DNA formed from irradiated and unirradiated complementary single strands: (A) T_m calculated from the change in mean length after irradiation; (B) T_m measured after irradiation of the deoxy(C-A) sequence; and (C) T_m measured after irradiation of the deoxy(T-G) sequence.

lowering of the T_m is considerably greater than that anticipated from the observed number of breaks introduced (Fig. 1). This must signify that a much greater number of bases are damaged than strand breaks are introduced. Similar conclusions have been reached by others from studies with natural DNA.

The known base sequence of the model compounds makes it possible to investigate the effects of base sequence on DNA radiation sensitivity. In the experiment depicted in Fig. 1, the double-strand DNA-like molecule formed from the irradiated C-A sequence and unirradiated T-G was found to be less stable than that formed from irradiated T-G and unirradiated C-A. In this case, the effect is probably due to preferential alteration by radiation of the C bases in the C-A sequence and of the T bases in the T-G sequence, plus the fact that G-C base pairs uniting the two strands are stronger than A-T base pairs. (More of these will remain after the T-G sequence has been irradiated than when the irradiated sequence is C-A.) Studies in which the model compounds have different sequences may reveal additional causes of the differences in radio-sensitivity between sequences.

The Influence of Base Sequences in Synthetic DNAs
on Transcription by RNA Polymerase

[D. A. Smith and A. M. Martinez]

The regulatory regions of a DNA must have features which allow their recognition by specific proteins (e.g., proteins involved in DNA replication and RNA transcription and nucleases involved in DNA repair). *In vitro* transcription of synthetic DNAs has provided important information on the type of base sequences which might be required for (1) binding of the enzyme to DNA; (2) initiation of RNA synthesis; (3) propagation of RNA chains; and (4) termination of the RNA chain. Hazardous agents which damage DNA could prevent normal transcription of a gene or allow transcription of portions of the DNA molecule which are normally not transcribed (Fig. 2).

The experiments described here deal with the transcription of synthetic DNAs containing deoxyinosine in place of deoxyguanine and illustrate that the stability of a DNA molecule can influence the kinetics of transcription by the RNA polymerase

from *Escherichia coli*. The kinetics of transcription of four repeating trinucleotide polymers, synthesized using either dGTP or dITP, and the other three deoxyribonucleoside triphosphates and their respective T_m values in 0.05 M Na^+ are given in Table I. It can be seen that deoxyinosine-containing polymers have a much lower T_m than their respective deoxyguanine counterparts; transcription by the RNA polymerase is 2- to 13-fold greater. It is likely that the RNA polymerase is able to separate more easily the double-stranded deoxy-nucleotide polymers containing deoxyinosine than the ones prepared using deoxyguanine, which allows elongation of the RNA strands to continue.

It has been suggested that the dynamic structure of DNA [i.e., the opening and closing (breathing) of portions of the DNA helix] may be important in its recognition by the RNA polymerase for transcription of a particular gene. Preparation of other synthetic DNAs, with known nucleotide sequences, should provide valuable tools for further studies of chemical agents which react with DNA and

Fig. 2. RNA polymerase transcription of a DNA chain.

TABLE I
TRANSCRIPTION OF DEOXYINOSINE- AND DEOXYGUANINE-
CONTAINING REPEATING TRINUCLEOTIDE POLYMERS BY THE
RNA POLYMERASE FROM *ESCHERICHIA COLI*^a

DNA Template	T _m (°C) (0.05 M Na ⁺)	Total Ribonucleoside Triphosphate Incorporated (nmoles)
d(AAC) _n · d(GTT) _n	75.5	11.10
d(AAC) _n · d(ITT) _n	54.0	86.70
d(ACT) _n · d(AGT) _n	69.5	32.40
d(ACT) _n · d(AIT) _n	50.0	67.70
d(ATC) _n · d(GAT) _n	73.5	34.66
d(ATC) _n · d(IAT) _n	51.5	61.84
d(AGC) _n · d(GCT) _n	91.1	6.02
d(AIC) _n · d(ICT) _n	51.5	78.16

^aEach reaction mixture contained 40 mM Tris-HCl buffer (pH 8.0), 4 mM MgCl₂, 1 mM MnCl₂, 12 mM 2-mercaptoethanol, 200 nmoles of the ribonucleoside triphosphates, 100 mono-nmoles of each polymer as template, and 100 units of RNA polymerase in a final volume of 1 ml. The reactions were incubated at 37°C for 1 h, and the incorporation of [¹⁴C]rCTP into acid-insoluble material was determined.

prevent these recognition sites from occurring or cause other recognition areas to appear, thereby stopping the synthesis of normal messenger RNA and allowing non-functional messenger RNA to be transcribed.

Organization of DNA in the Mammalian Nucleus during the Cell Division Cycle

[C. E. Hildebrand, R. A. Tobey, L. S. Gurley, and R. T. Okinaka]

Assessment of potential biological hazards associated with new energy technology can be facilitated by studying the mechanisms governing the interaction of energy by-products with living cells. Cellular DNA provides a blueprint function in that it maintains parent-progeny genetic continuity and supplies the source of information for synthesis of cellular components required for normal cell growth, division, and differentiation. Hence, interaction of energy-related chemical (organic and inorganic) and/or physical (heat, electromagnetic radiations) agents could result in cell death, as well as elicit carcinogenic, mutagenic, teratogenic, or other abnormal physiological responses. In eucaryotic cells, the DNA is compartmentalized in the nucleus where packaging and genetic expression are regulated by specific proteins (both histones and non-histone proteins). The intimate protein-DNA associations suggest that damage to DNA is very likely modified and/or mediated by the associated proteins. The major thrust of this activity is directed toward identifying the determinants of DNA organization in the nucleus, measuring changes in DNA organization in various stages of cell proliferation or growth arrest, and relating structural changes to changes in cell function.

Studies performed within this activity can be classified into three major areas: (1) attachment of nuclear DNA to a supramolecular nuclear structure (presumably the nuclear envelope); (2) identification of a morphologically and biochemically defined protein network which extends throughout the nucleus and to which DNA is attached; and (3) characterization of changes in the intranuclear organization of DNA (chromatin) during the growth cycle. In all of these studies, the mammalian tissue culture cell line CHO (derived from the Chinese hamster) was used. The existing wealth of information on the cell proliferation cycle and on protein, RNA, and DNA metabolism in CHO developed in this Laboratory has provided an ideal system in which results from this activity can be interrelated with studies on DNA

replication and expression and on histone synthesis and modification. In addition, considerations of DNA organization during the cell cycle are relevant to studies of the cell-cycle-specific actions of drugs under investigation in this Laboratory.

In earlier studies, we found that a small fraction (approximately 10%) of total nuclear DNA was specifically bound to a phospholipid-containing nuclear structure. Further investigations showed that this bound fraction of DNA contained a large portion of the modified cytosine (5-methyl-cytosine) residues when compared with unbound DNA. In addition, in a synchronized cell population, the fraction of bound DNA increased from 10% to 22% during the G₁ phase of the cell cycle, with the increase beginning approximately 2 h prior to the S phase. This change in DNA organization correlates in time with the phosphorylation of lysine-rich histone fraction fl. During the S phase, most (75%) of the replicating DNA was localized in the bound DNA fraction but was subsequently transferred to the unbound fraction. In view of the concurrence by several laboratories that replicating DNA is not localized at the nuclear envelope, we pursued the notion that DNA may be associated with a supramolecular nuclear structure which not only included the nuclear periphery but also permeated the central portion of the nucleus. Such a structure was found and has been characterized morphologically and biochemically. The morphology of the residual nuclear matrix prepared by extensive RNase and DNase digestion and 2 M NaCl extraction of isolated nuclei is compared with an intact nucleus in Fig. 1. The matrix is composed almost totally of protein, of which there is one major class and several minor species detectable by SDS-gel electrophoresis.

In other studies, an agent (the polyanion, heparin) which interacts predominantly with the histone component of chromatin has been found useful as a probe of cell-cycle-specific changes in nuclear organization. Preliminary results suggest that histones become less accessible in a well-defined manner to polyanions as cells traverse the proliferation cycle.

Further studies of changes in DNA organization in different stages of the cell cycle are being pursued using other probes. In addition, an agent (x irradiation) has already been found to produce an

Fig. 1. Electron micrographs: (A) thin-section of isolated Chinese hamster cell nucleus (magnification X16 000); (B) thin-section of nuclear matrix prepared from Chinese hamster cell nuclei [magnification X13 000; note the similarity in size of the nucleus and nuclear matrix and the distribution of protein (dark areas) in the matrix]; and (C) whole nuclear matrix prepared by critical point drying (magnification X10 500).

effect on chromatin structure detectable by the heparin probe. These studies will provide a clearer picture of how DNA is organized in the nucleus and consequently will be useful in assembling a conceptual framework for defining mechanisms by which energy-related chemical and physical agents exert an influence on DNA in different states of organization.

Pollutants and Genetic Information Transfer

[R. L. Ratliff and D. A. Smith]

Genetic expression is capable of amplifying individual molecular events into macroscopic disorders. Agents which perturb the fidelity of DNA replication or RNA transcription must produce harmful effects in biological systems. Therefore, one of the main concerns in evaluation of the health aspects of alternate modes of energy production must be to identify and quantify those associated agents which interfere with the maintenance and transfer of genetic information from DNA to DNA and from DNA to RNA.

This research aims to take advantage of the defined model DNAs available in this Laboratory to study in cell-free systems the effects of energy-related pollutants on the maintenance and expression of genetic information. Model systems of this type have been used to provide much of our present understanding of the mechanisms of genetic information transfer and are probably the system of choice with which to study the molecular mechanisms of deleterious agents which interact with DNA. The properties of a DNA molecule are specified by its unique base sequence; however, the large size and varied base sequence of naturally occurring DNAs make them impractical tools for the study of the influence of sequence on DNA properties and function. The success of this approach lies in the use of simplified model DNAs and highly purified enzymes. Model DNAs with repeating nucleotide sequences are first prepared by a combination of laborious organic chemical procedures and enzymatic polymerization. Once prepared, a continuous supply of the unique polymer is maintained with relatively simple enzymatic techniques.

The power of model DNAs in discovering important new mechanistic insights is exemplified by our recent results on the effects of x irradiation on three isomeric polymers, each containing equal

TABLE I
POLYMER OF ORIGINAL SIZE
REMAINING AFTER X IRRADIATION^a

Dose (krads)	Percent of Recovered Radioactivity			
	$dA_n \cdot dT_n$	$d(A-T)_n \cdot d(A-T)_n$	$d(A-A-T)_n \cdot d(A-T-T)_n$	
10	96.5	35.8	85.1	
40	70.4	2.9	27.3	
80	26.9	1.9	7.6	

^aFractions of the three polymers of the same average size were irradiated and then passed through a calibrated gel exclusion column. The results are expressed as the percent of recovered polymer which still appears to have its original size.

amounts of adenine and thymine but having different base sequences. Fractions of the polymers, $dA_n \cdot dT_n$, $d(A-T)_n \cdot d(A-T)_n$, and $d(A-A-T)_n \cdot d(A-T-T)_n$, of the same average length were isolated and subjected to x irradiation. The polymers were then passed through calibrated gel exclusion columns in order to measure breakdown of the polymers. It can be seen from Table I that there was a great difference in the radiation-induced degradation of the three polymers. Likewise, when the irradiated polymers were assayed as templates for RNA polymerase, a similar difference was seen. Thus, base sequence appears to play an important and hitherto unrecognized role in radiation damage to nucleic acids.

A very active current effort is a study of the effect of various heavy metal ions on the fidelity with which nucleic acid polymerases copy defined DNA templates (i.e., whether heavy metals can cause these enzymes to make mistakes). We have shown previously that x irradiation of RNA polymerase greatly increases the error frequency of this enzyme. Other current work includes a study of the binding of various chemical agents to DNA as a function of base composition and sequence and the preparation and characterization of new model DNAs.

In addition to their special usefulness in studies of pollutant effects, model polymers provide new information about the influence of base sequence on DNA properties. Publications in the past year include descriptions of the preparation and properties of a new model DNA, buoyant density studies considered as sequence-dependent properties, and studies of nucleic acid conformation as measured

by the technique of circular dichroism. Other publications include descriptions of the initiation of RNA synthesis by covalent attachment to DNA oligomers and to irradiated DNA.

Detailed knowledge of the mechanisms of repair of damage to DNA is relatively meager, especially in mammalian systems. This is an important area where the use of specially prepared models promises to be particularly rewarding.

Effects of Nuclear and Non-Nuclear Pollutants on Gene Expression in Mammalian Cells: The Necessity of an Integrated, Broad-Based Program of Basic and Applied Research

[M. D. Enger, E. W. Campbell, P. Ritter, C. E. Hildebrand, and J. L. Hanners]

Definition of the effects of toxic pollutants on gene expression in mammalian cells requires, first, a definition of the mechanisms involved in such expression and, second, an application of the knowledge and techniques developed in the course of such basic research to analysis of the nature and extent of damage due to such agents. As can be seen from Fig. 2, the complexity of the system that comprises the mechanisms of genetic expression is such that a broad-based analytical program is essential. Also, the need for further information about most, if not all, components of the system and for the refined analyses to study each makes an extensive basic research program an essential adjunct to a successful description of the effects of toxicants thereon.

The system, as presently understood, is shown in Fig. 2, along with an enumeration of possible sites at which expression may be altered or prevented. Many chemical and physical agents cause direct damage to DNA, the genetic information. Such damage (1) may be expressed simply as altered messenger RNA which, in turn, is translated into defective protein (enzymes). Mammalian DNA is extensively associated with proteins, forming chromatin. Alterations in the nature of such associations (2) may change the pattern of gene expression. Essential genes may now be covered, or genes causing cellular changes such as tumorigenic transformation may be uncovered. Items (1) and (2) are the subject of several interacting LASL programs.

This program is concerned with learning more about processes (3-9) and using the techniques

Fig. 2. Possible sites of alterations in genetic expression due to exposure of mammalian cells to toxic agents. (1) Transcription of damaged DNA as altered or incomplete messenger precursor and products. (2) Alterations in chromatin structure may block expression of vital genes. (3) Normal transcription produces a high molecular weight precursor. (4) Some precursors are normally degraded. (5) Others are selected for trimming, modification, and association with proteins to form pre-mRNP. (6) Selected messenger nucleoproteins are transported to the cytoplasm. Alteration of specificity or function of items (4-6) may alter gene expression as effectively as DNA damage. (7) Not all cytoplasmic mRNPs, or informosomes, are active in the sense that they can participate in polysome formation. Again, a mechanism operates with a specificity and efficiency whose alteration may change gene expression. (8) Polysome assembly involves interaction of a number of components potentially affected by toxicants. (9) Polysome function may be specifically or generally affected.

applicable to such studies and the derived understanding to define the effects of ionizing radiation, as well as pollutants (such as heavy metals) associated with non-nuclear energy production. Mammalian DNA is expressed (3) as a high molecular weight precursor (pre-mRNA) to messenger RNA (the intermediary between DNA and protein). Some of these precursors are degraded (4); others (5) are extensively modified by trimming, methylation, polyadenylation, and association with proteins to form pre-mRNP. Some pre-mRNPs are then selected (6) for transport to the cytoplasm. The selectivity of processes (4-6) and their importance as determinants of gene expression are illustrated by the fact that 5 to 10 times more genetic information exists in RNA of the nucleus

than in the cytoplasmic RNA. Thus, agents affecting the efficiency or selectivity of such processes would have as great an effect on gene expression as those directly affecting DNA.

Once in the cytoplasm, messenger nucleoprotein (informosomes) must associate with ribosomes, factors, and appropriately activated tRNA in the end products of gene expression. Our studies have indicated that some informosomes cannot associate with such factors in the absence of some activating or derepressing process (7). Some information appears to be sequestered at this stage. We have found that a late effect of ionizing radiation is an alteration in the partitioning of messenger between informosomes and polysomes.

It is evident that a number of processes are involved in the assembly of polysomes (8). Again, specificity and selectivity are involved. For example, initiation factors may discriminate against certain messengers. Alterations in the fidelity of tRNA charging (with amino acids) may produce an end effect equivalent to that effected by direct damage to the genome. General or selective effects on the translation process itself (9), once polysomes are formed, may also occur. Investigation of this system has allowed us to contribute to an understanding of the effects of ionizing radiation and chemical toxicants on the integrated system and, we believe, represents a unique contribution.

Structure of Nucleic Acids

[A. G. Saponara, M. D. Enger, and J. L. Hanners]

Toxic environmental agents will affect cells by interfering with vital functions. The ribonucleic acids (RNAs) are one of the important classes of molecules which must be continually synthesized by cells and, therefore, are a potential target. A number of known vital functions are mediated by ribonucleic acids. Thus, there are three classes of RNAs which are required for a cell to express its genetic information. These are the messenger RNAs which carry the actual information for the synthesis of proteins, the four ribosomal RNAs which are constituents of the particles performing the assembly process, and the transfer RNAs which contribute to the assembly as adaptors, properly positioning the amino acids to be joined into those unique sequences dictated by the messenger.

In addition to these RNAs whose functions are well established, another class of RNAs (LMM-RNAs) of unknown function has recently been discovered, in part by work conducted at the Los Alamos Scientific Laboratory. The LMM-RNAs have since been shown to be common constituents of higher organisms, and their ubiquity argues that they also serve a vital function. Most, perhaps all, of these RNAs share in an unexplained, circuitous, synthetic pattern, being initially synthesized as larger molecules which are subsequently pruned to their mature sizes and often modified at one or the other of their ends or at specific internal sites. Some of these modifications are unique to a particular class of RNA. Thus, mammalian messenger RNAs have an unusually linked

7-methyl guanosine cap at one end, a modification which appears to be required for messenger utilization. This peculiar type of cap is also found on three of the LMM-RNAs. In this case, however the terminal nucleotide is a trimethyl guanosine. We have found another provocative modification on the small subunit ribosomal RNA of mammalian cells. Here, a single uridine of this 2000-nucleotide-long chain is rearranged and multiply modified, resulting in a hybrid amino acid nucleotide derivative. One can estimate that at least two and possibly three enzymes have evolved for the specific purpose of generating this particular modified base. Again, although no function is currently known, scientific intuition strongly suggests that this level of mechanistic commitment is associated with a commensurate functional significance.

We have sketched some of the aspects of nucleic acid synthesis and function which are of interest at the LASL. This represents only a small part of the voluminous data dealing with RNA metabolism. Our purpose is to point out features of the nucleic acids which make them an ideal end point for physiological effects studies. Taken collectively, RNAs represent a vital class of cell macromolecules, their synthesis, modification, and utilization requiring a large share of cell total commitment of energy and enzymatic capability. Interference at any of a large number of sequential steps could result in the accumulation of inactive or functionally defective RNAs which will disrupt the normal cell.

Kinetic and Biochemical Effects of Hazardous Agents on Cell Proliferation Capacity

[R. A. Tobey, M. S. Oka, H. A. Crissman, and L. T. Ferzoco]

As new energy technologies are developed and put into operation, the number of associated, potentially hazardous substances will be greatly increased. To obtain an idea of the magnitude of the risk and to establish parameters to be monitored in humans involved in these technologies, it would be helpful to assay the effects of agents in simple model systems designed to act as predictive indicators of human response.

A system has been developed at the Los Alamos Scientific Laboratory which allows production of both proliferating and non-proliferating cell

populations in tissue culture, mimicking these classes of somatic cells in humans. An early indication of exposure to toxic levels of hazardous agents is a rearrangement of cells in the *division cycle* (i.e., the sequence of biochemical processes that allows a cell to double its components in preparation for division into two equal daughters). Localization of cells within the division cycle is accomplished by measuring the content of genetic material (DNA) in individual cells, with late-cycle cells having twice as much DNA as cells in early stages, etc. Rapid analytical techniques involving fluorescent DNA stains have been developed to facilitate measurement of cells in the cell cycle. These techniques allow resolution of even small population changes. Results to date suggest that the specific type of rearrangement of cells in the division cycle is characteristic for the mechanism of biochemical action of the agent tested (i.e., agents with similar types of biochemical action produce similar types of kinetic changes).

As an integral part of the assay program, we are also concerned with mechanisms of action of toxic agents, with special emphasis on the biochemical processes affected to yield the division cycle redistributions. Since transformation of normal cells by carcinogenic agents to the neoplastic state involves a loss in proliferation control capacity, we are investigating simultaneously a number of processes involved in preparation for cell division to better understand the basic mechanisms of growth control operative in animal cell model systems.

Fig. 3. Temporal arrangement of biochemical events in the mammalian cell division cycle.

Our system for reversibly arresting cell populations once again is a highly useful tool in that (1) we are able to investigate differences in cycling and non-cycling cultures and (2) we are able to synchronize populations so that essentially all the cells in the culture perform the same biochemical task at the same time. As might be anticipated, proliferation capacity depends on a complex series of coordinated events. Figure 3 summarizes the results obtained at LASL. It is highly significant that even cells grown in the artificial environment of tissue culture are subject to specific regulatory processes which ensure an orderly sequence of operations as cells prepare for DNA replication and division.

Histone Phosphorylation: Its Role in Cell Proliferation

[L. R. Gurley, J. D'Anna, R. A. Tobey, R. A. Walters, and J. G. Valdez]

The formation of chromosomes and the replication of cells by mitosis are fundamental processes in all proliferating eucaryotic life. In cancer cells, these processes are under less restricted control than in normal cells, resulting in malignant tissue growth. Therefore, an understanding of the control of these processes is necessary if the mechanisms of carcinogenesis are to be determined. Experiments in this Laboratory have shown that histone phosphorylation is involved in cell proliferation. If a positive role for histone phosphorylation in chromosome structure and cell division could be demonstrated, it would be potentially important to a carcinogenesis program. This impetus has resulted in the design of experiments to identify the various histone phosphorylation events and to correlate them with chromosomal structural changes.

Using synchronized cell cultures, we have determined the cell-cycle schedule of histone phosphorylation. Figure 4 illustrates diagrammatically this schedule. In non-dividing cells (G₁-arrest), only histone f2a2 phosphorylation occurs. However, when these cells are allowed to proliferate, histone f1 phosphorylation begins in mid-G₁. When these cells enter the chromosomal replication phase (S), an additional f1 phosphorylation occurs. Structural studies on this histone have determined that these interphase phosphorylations occur only on serine residues in the C-terminal end of the f1 molecule.

Fig. 4. The histone phosphorylation schedule in the line CHO Chinese hamster cell cycle. The phases of the cell cycle for proliferating cells are G_1 (the pre-DNA synthetic phase), S (the DNA synthetic phase), G_2 (the pre-cell division phase), and M (the cell division phase which is broken down into prophase, anaphase, and telophase). G_1 -Arrest indicates the position non-proliferating cells occupy in the cell cycle. The times during which histones f1, f2a2, and f3 are phosphorylated are indicated by the shaded bands.

When the cells enter mitosis (the final phase of their life cycle), a third f1 phosphorylation event occurs. In addition, histone f3 is phosphorylated for the first and only time during mitosis. Structural studies on tryptic digests of f1 have shown that the mitotic phosphorylations occur at sites which are different from the interphase phosphorylation sites. These mitotic sites include the phosphorylation of both serine and threonine residues in the N-terminal end of the molecule, as well as on serine and threonine residues in the C-terminal end. Thus, we have demonstrated that f1 phosphorylation is an ordered process, occurring at different specific sites during specific times in the cell cycle. This information is important because it will enable us to identify the specific enzymes responsible for the various phosphorylations of histones during the cell cycle.

Initial attempts to correlate these cell-cycle-specific histone phosphorylation events with chromosomal structural changes have been successful. By measuring the ability of heparin to complex with chromatin-bound histones, we found that chromatin structural changes occur during the G_1 phase. Cell-cycle correlations were found to exist between the

G_1 phosphorylation of histone f1 and the association of a DNA-lipoprotein complex with magnesium-sarcosyl crystals. This has led to the speculation that the G_1 phosphorylation of histone f1 may alter the structural configuration of chromatin in such a way as to facilitate cell proliferation. Chromatin structural changes presumed to be necessary for DNA replication may also involve the f1 phosphorylation event initiated specifically at the beginning of S phase. The coincidence of mitotic f1 and f3 phosphorylation with the condensation of chromosomes at mitosis strongly suggests that these two phosphorylations are involved in the chromatin structural changes necessary for orderly cell replication.

Effects of Energy-Related Physical and Chemical Carcinogens and Mutagens on Chromatin Structure and Function

[G. F. Strniste, G. R. Shepherd, J. M. Hardin, and J. C. Rall]

A concurrent understanding of the environmental impact of various energy-related agents created by new energy technologies is a necessity in order to ascertain the biological risks involved and to establish acceptable tolerance levels in the environment for the multitude of potentially harmful pollutants resulting as the technologies develop. To assess accurately the impact of various agents on human health, it is imperative that basic laboratory animal studies be supplemented with more molecular-oriented approaches in order to establish a more conceptual understanding of the biological mechanisms affected by such potentially detrimental agents. It is with this understanding that the processes of extrapolating laboratory results to man should be facilitated.

The interaction of various physical or chemical agents with biological systems can lead to serious biological end points, including mutagenesis, carcinogenesis, and cell death. It is currently believed that these agents interact with the DNA of the target system, resulting in aberrations to the genetic apparatus of the cell. In eucaryotic cells, the DNA is heterogeneously complexed as chromatin with a variety of acidic and basic proteins which contribute to the structural organization of chromatin and to genetic regulation. Since the complexity in organization of chromatin may be expected to modify the response of eucaryotic DNA to the attacks

TABLE II

FORMATION OF PROTEIN-DNA ADDUCTS IN CHROMATIN WITH ULTRAVIOLET LIGHT
AS DETECTED BY SEPHAROSE 4B COLUMN CHROMATOGRAPHY^a

UV Light Fluence (Jm ⁻²)	¹⁴ C-Lysine (% total cpm)	¹⁴ C-Leucine (% total cpm)	¹⁴ C-Tryptophan (% total cpm)
0	0 ^b	0	0
2928	1.6	1.0	2.5
5856	3.2	2.0	4.7
8784	4.7	2.7	7.3
11 712	6.4	3.9	9.7

^aThe protein components of chromatin were labeled *in vivo* with either ¹⁴C-lysine, ¹⁴C-leucine, or ¹⁴C-tryptophan. Solutions of isolated chromatin were exposed to several fluences of uv light and incubated in 2 M NaCl, 0.1% sodium lauryl sarcosinate, prior to elution through a Sepharose 4B column. The data represent the percent of the total cpm which elutes coincident with the DNA (void volume), indicating protein-DNA complexes (adducts) which are resistant to dissociation in high salt and detergent.

^bControl values of 1.5–2.5% of the total cpm for non-irradiated chromatin have been subtracted from each point.

Fig. 5. Schematic drawing showing the formation of protein-DNA adducts in chromatin. Chromosomal proteins are involved in maintenance of the physical structure and in the genetic regulation of DNA in eucaryotic cells. The interaction of various chemical and physical agents with chromatin can result in intercomponent covalently linked adducts. Such alterations in chromatin could interfere with the normal genetic processes of the cell, thus possibly contributing to cell mutagenesis, carcinogenesis, premature aging, and death. The capacity of the cell to recognize and to repair protein-DNA adducts in chromatin is not known.

by chemical or physical agents, this investigation was initiated in an attempt to define structural changes induced in mammalian chromatin by energy-related hazardous agents and to relate these changes with fundamental physiological changes in cell properties. Isolated chromatin (*in vitro* analysis) or cultured cells (*in vivo* analysis) will be exposed to various analogs of energy-related agents, and the induced lesions in the chromatin, including mono- and difunctional adducts as well as intercomponent cross links, will be characterized and quantitated and attempts made to correlate the various induced modifications in chromatin with cell survival or transformation.

Results from our Laboratory have shown that, in ultraviolet light- (analog of sunlight) irradiated chromatin, there exists a dose-dependent response in the formation of intercomponent, covalently linked adducts between chromatin DNA and protein. Three independent methods of analysis have been utilized in detecting uv light-induced stable protein-DNA adducts: (1) a membrane filter assay in which DNA associated with protein after high salt-detergent treatment is retained on the filter; (2) a Sepharose 4B column in which protein associated with DNA after high salt-detergent treatment elutes coincident with the DNA; and (3) a cesium chloride density gradient equilibrium assay in which protein-DNA complexes resistant to dissociation by high salt-detergent band at densities other than the native banding positions for either the DNA or the protein. When chromatin is exposed to differing conditions of pH, ionic strength, or divalent metal ion concentration, the quantity of adducts formed upon uv irradiation varies. It appears as if maximum adduct formation is obtained under conditions which optimize chromatin condensation. Using chromatin preparations in which the protein components have been radioisotopically labeled with different amino acid precursors, it appears as if linkage of protein to DNA is not random (see Table II). There appears to be some mode of specificity in the linkage of various chromosomal histone and non-histone proteins to the DNA. In current experiments, we are attempting to define qualitatively which proteins are involved in adduct formation using chromatin irradiated in solution and chromatin isolated from irradiated tissue culture cells.

Adduct formation between the protein and DNA components of chromatin could have catastrophic effects on the structure and function of the basic genetic machinery of the cell (Fig. 5). Using the various assays we have developed, we are currently prepared to analyze a variety of other potentially harmful energy-related agents in order to assess their role as possible candidates in the generation of adducts in chromatin. Future experiments are also being designed to examine the capacity of a cell to recognize and repair such induced lesions. Evidence that X Irradiation Inhibits DNA Replicon Interaction in Chinese Hamster Cells

[R. A. Walters, C. E. Hildebrand, and J. L. Hanners]

An understanding of the metabolic effects of ionizing radiation on living cells is required from the standpoint of either use in radiotherapy or evaluation of possible consequences of accidental release of nuclear materials into the environment. Deoxyribonucleic acid (DNA) assumes a primary role in this regard as it is the heritable genetic material of the cell. It is known that ionizing radiation damages cellular DNA and that much of this damage can be repaired. However, very little is known of the mechanistic aspects of possible effects of ionizing radiation on DNA synthesis (e.g., replication).

DNA replication in each mammalian cell proceeds from many thousands of segments of DNA, each segment of which is termed a replicon. DNA synthesis (or replication) begins in the interior of the replicon, and synthesis proceeds in both directions from the origin. The DNA is replicated in very small pieces (e.g., chain elongation), after which the DNA chain is ligated to form a larger piece of DNA, this process continuing until synthesis of the replicon is complete. The replicon must then be joined or integrated into the very large, high molecular weight DNA characteristic of the bulk of cellular DNA. Thus, it is possible that radiation could affect DNA synthesis by (1) inhibiting initiation of replicon synthesis, (2) inhibiting chain elongation, or (3) inhibiting ligation into high molecular weight DNA. To determine which of these possibilities contributes to inhibition of DNA synthesis after x irradiation, the following experiment was performed.

Fig. 6. (A) The size distribution of newly synthesized DNA from an alkaline sucrose density gradient (note the relative paucity of small DNA pieces and the overabundance of larger DNAs in the irradiated sample). (B) The dose-response of inhibition of synthesis of total unfractionated DNA (note the biphasic response with a radiosensitive portion < 800 rads and a radioresistant portion > 800 rads).

Chinese hamster cells were x-irradiated with 800 and 1600 rads, and the DNA was radioactively pulse-labeled from 20 to 30 min post-irradiation. The newly synthesized DNA was analyzed by alkaline sucrose density ultracentrifugation, which allows one to examine the size of the newly replicated DNA. An example of the results is shown in Fig. 6A. It can be seen that the irradiated sample contains much less small DNA, with a relative overabundance of larger DNA pieces. Knowing the rate of DNA replication and the amount of radioactivity incorporated into the small and large DNA pieces in the alkaline gradient, we can show that any replicon that was either very near to or in the process of initiation of DNA synthesis was inhibited from initiation by irradiation, while those replicons that were already in the process of synthesis at the time of irradiation were inhibited very little in completion of synthesis and subsequent ligation (joining) into high molecular weight DNA. We can now account for the well-known, classical, biphasic dose-response curve of x-ray inhibition of total DNA synthesis shown in Fig. 6B. The radiosensitive portion (less than 800 rads) is the result of inhibition of replicon initiation, while the radioresistant portion (> 800 rads) is due to inhibition of chain elongation and/or subsequent ligation into high molecular weight DNA. This is the first qualitative and quantitative demonstration of the mechanism of ionizing radiation-induced inhibition of DNA synthesis.

Mathematical Models of the Immune Response

[G. I. Bell and A. S. Perelson]

The immune response is a primary defense mechanism against pathogenic microorganisms and some environmental chemicals. Moreover, responsiveness is often perturbed as a result of environmental insults, and indeed the clinical manifestations of exposure to pollutants are often associated with or caused by the immune response, as in allergic reactions or tumor induction through persistent irritation. Finally, the immune system may have an important role in suppressing nascent tumors or metastases. In view of these considerations, it is apparent that improved understanding of the function and control of the immune response is an important part of the long-range program to assess the effects of environmental pollutants and radiation on human health. The present program is designed to apply the modeling techniques of mathematical physics to key problems in immunology. Some of this work is supported by other ERDA and NIH funding.

During the preceding year, we developed a theoretical model of the plaque assay in which possible antibody secreting cells are overlaid on a lawn of sheep red cells. In the presence of complement, an area of lysis surrounds each cell secreting the appropriate antibodies; therefore, the assay is widely used for enumerating antibody secreting cells. We showed that, in addition, the plaque assay can be used to obtain quantitative antibody secretion rates and antigen-antibody binding constants. During the current year, we refined these theoretical models and applied them to a new assay developed by Alistair Cunningham for identifying cells in a clone producing antibodies of altered specificity. Mixed lawns of, say, sheep and goat red cells are used in this technique, and plaques of three different morphologies are observed: clear plaques in which both kinds of red cells are equally lysed, cloudy plaques in which only one kind is lysed, and sombrero plaques in which unequal radii of lysis are seen for the two populations of red cells. Cunningham observed plaques of altered morphology within clones and interpreted these as mutant cells secreting antibodies of altered specificity. From these observations, he concluded that much antibody diversity arises during antigen-driven proliferation of lymphocytes.

Fig. 7. Hemolytic plaques of altered morphology may be indicators of somatic mutation.

Because of the importance of this unconventional observation, we have extended our theory to plaques with two types of red cells. In principle, plaques of altered morphology can arise from altered antibody

secretion rates. However, we concluded that, under the conditions of Cunningham's experiments, plaque morphology is insensitive to secretion rate and that altered morphology is a valid indicator of altered antibody specificity. Additional experiments to confirm this interpretation have been suggested. While this assay has so far been used to indicate a surprisingly high incidence of natural mutations, it is possible that it could be developed to test for environmental mutagenesis (Fig. 7).

In addition, we have continued our theoretical studies of the binding of multivalent antigens to antibody-like receptors on lymphocytes, which is believed to be the key antigen-specific step in triggering an immune response. We have evaluated the inhibition of antigen binding by free hapten and shown under what conditions this inhibition is an effective indicator of antigen-receptor affinity. An improved model of the kinetics of antigen-receptor lattice formation (patching) has been developed, and we have investigated the dependence of patching times on antigen valence. We believe that these models will be valuable for understanding why antigen binding to lymphocytes sometimes results in cell proliferation and antibody secretion and, under other conditions, to a more or less permanent paralysis of responsiveness.

Properties of the Cell Surface

[P. M. Kraemer and M. Da Silva]

The ultimate consequences of the exposure of humans to mutagenic, carcinogenic, or teratogenic agents are a function of the surface properties of individual cells in two ways. First, the chemical nature of the cell surface determines whether and in what manner exogenous agents are permitted to enter the cell. For instance, specific receptors on the surfaces of some cells determine whether metal ions transported by serum transferrin are deposited on those cells. Second, there is now

Fig. 1. Diagrammatic view of heparan sulfate of the cell surface. This material appears to be important in regulation of the exposure of the cell surface of all animal cells. Heparan sulfate molecules consist of a protein portion, anchored in the cell surface membrane, to which are attached long sugar chains bearing numerous acidic groups.

much indirect evidence that the behavior of cells that have been altered by such mutagenic, carcinogenic, or teratogenic agents involves changes in the chemical and physical structure of the cell periphery.

Different types of cells have specific cell surface structures according to their particular functions. In addition, we have discovered that the mucopolysaccharide, heparan sulfate, is a ubiquitous component of the cell surface of animal cells and, therefore, have proposed that this entity serves a general life function (Fig. 1). In particular, our studies suggest a role for cell surface heparan sulfate in cell division, since a large portion of the molecules suddenly detach themselves from the cell surface just prior to mitosis. This "desquamation" event concerns heparan sulfate alone; numerous other cell surface entities, including specific receptor sites, are not involved. On the contrary, evidence from a number of laboratories shows that, at the time of heparan sulfate release, these other cell surface sites become more exposed. Consequently, we have proposed that heparan sulfate of the cell surface may serve as a non-specific modulator of the accessibility of various cell-specific surface sites to the external environment. We anticipate that studies of the metabolism and control of cell surface heparan sulfate will be of some interest to questions concerning the consequences of exposure, at the cellular level, to mutagenic, carcinogenic, and teratogenic agents.

Rapid Karyotype Analysis with Flow Systems

[L. L. Deaven, P. C. Sanders, and L. T. Ferzoco]

Ample evidence is available to demonstrate that physical, chemical, and biological agents in the environment can induce both chromosome damage and genetic mutations. In mammalian cells, there is good correlation between mutagenesis and chromosome damage; however, some mutations occur in the absence of visible chromosome aberrations. Chromosome analysis may prove to be one of the best screening systems for the mutagenic potential of environmental pollutants; however, more information is needed regarding the precise relationships between chromosome changes and mutagenesis, and methods for chromosome analysis must be made less subjective and time-consuming than those available currently.

Accordingly, we have been examining the karyotypes of mutant cell populations isolated in the somatic cell genetics effort in this Laboratory. These karyotype analyses include chromosome banding methods, which allow more precise identification of chromosome rearrangements than classic techniques, and rapid analysis using flow microfluorometric techniques. Chromosome banding results in the induction of a series of dark and light bands of varying sizes and intensities along the length of a chromosome. These bands are highly reproducible in normal chromosomes, and examination of the patterns permits the identification of missing or rearranged segments which could not be identified by microscopic measurements (Fig. 1).

Flow-systems development has undergone gradual improvement in the Biophysics and Instrumentation Group at this Laboratory during the past few years, and a number of instruments capable of analyzing morphologic and fluorescent properties of particles in liquid suspension are currently available. Briefly, cells or subcellular particles are stained with a fluorescent dye specific for one or more biochemical constituents and run through a laser beam. The resulting pulses of fluorescent light are measured and recorded, providing a measure of the amount of DNA or protein in a given particle.

Karyotype analysis is accomplished in the flow systems by breaking metaphase cells to release the chromosomes, staining them with DNA-specific fluorescent stains, and running the stained chromosomes

Fig. 1. Chromosomal banding patterns in a cloned line of Chinese hamster (line CHO) cells.

Fig. 2. Flow microfluorometric spectrum of chromosomes isolated from Chinese hamster ovary cells.

through the appropriate instrument. The analysis reveals a series of peaks and shoulders which correspond to the individual chromosomes present in the cell being studied (Fig. 2). Changes in the karyotype such as stable chromosome rearrangements would be reflected in the spectra by the appearance of new peaks, the increase or decrease in size of existing peaks, or the disappearance of some peaks. The typical sample size of these distributions is 50 000 or more chromosomes, allowing a statistical precision unobtainable by conventional karyotype analysis. The actual running time on the flow microfluorometer is several minutes.

We have examined four mutant clones of Chinese hamster (line CHO) chromosomes by the above procedures. One clone is identical to the parent line, while the other three show karyotype rearrangements

both by banding and flow microfluorometry. The accumulation of this type of data will allow us to characterize and calibrate the system in order to interpret data from cell lines containing random chromosome aberrations. In the latter case, aberration rates will have to be determined by increases in the number of random-sized chromosomes rather than in specific peak increases.

These results suggest that rapid karyotype analysis by flow systems is feasible and that it can be applied to chromosome aberration induction by toxic materials in the environment. Other potential uses of this technique are the isolation of quantities of single chromosomes for biochemical and immunological analysis and studies of chromosomal constitutions of malignant cells.

Biochemical Genetics

[B. J. Barnhart, S. H. Cox, R. T. Okinaka, and P. M. Kraemer]

We have initiated within the past year an effort designed to obtain conditional-lethal variants (mutants) of the mammalian cell line CHO which has been studied extensively in this Laboratory. We have succeeded in isolating from mutagenized cultures seven temperature-sensitive (t_s) sublines of CHO. These mutants do not form colonies at 39°C but grow normally at 34°C. Flow microfluorometric (FMF) analysis revealed that, when incubated at 39°C, the majority of cells in one of these t_s -mutant populations is blocked in phase G_2 or M of the cell life cycle (Fig. 1). Mutant sublines such as this one will be a valuable asset in research designed to elucidate the regulation of the cell

Fig. 1. DNA distributions obtained by FMF analysis of synchronized monolayers of CHO cells 10 h after release from hydroxyurea and simultaneous transfer from 34°C to 39°C: (A) a temperature-sensitive mutant population displaying a majority of cells blocked in the " $G_2 + M$ " portion of the FMF profile and (B) the parental wild-type population having the majority of cells in the " G_1 " phase of the cell life cycle. Abscissa: relative DNA content. Ordinate: number of cells ($\times 10^{-3}$).

life cycle. The use of mammalian cell t_s -mutants as a detection system for possible influences of polluting substances at the genetic level offers a simplified procedure facilitated by visual quantitation.

In addition to t_s -mutants, we have isolated two others which vary in rates of detachment with trypsin from the substratum. One of the detachment variants (DV-10) is more sensitive to trypsin so that 50% of the cells are detached after 2 min, while the parental wild-type line exhibits 50% removal by 7 min, and 50% of another variant (DV-1) is detached only after 16 min. The importance of these variants is that cell adhesion has been implicated by many workers as an important parameter, changes in which suggest alterations in the regulation of cellular growth rates, intercellular cAMP levels, cellular morphology, etc. Biochemical analysis of DV-1 has revealed that these cells completely lack the hyaluronic acid component of the complex carbohydrates of the cell periphery. Since hyaluronic acid has been suggested by some workers as the glue by which cells adhere to the substratum, we did not anticipate its absence from cells exhibiting enhanced adhesion. The lack of this component had no pleiotropic effects on growth rate, cAMP levels, cell morphology, or cell volume, all of which were normal. Sublines with altered cell surface biochemistry are also interesting with respect to cellular susceptibility to injury by chemical pollutants. The chemistry of cellular contact or entry by damaging agents might possibly be determined using mutants with an altered cell periphery.

This activity also includes the investigation of a microbial system. This work has included a study of the mechanism of ultraviolet light induction of a prophage and an investigation of cellular repair of replicating phage DNA irradiated with ultraviolet light at various times after phage induction in both lysogenic repair-proficient and repair-deficient strains of *Haemophilus influenzae*. Results of the prophage induction work suggest that induction occurs only when one ultraviolet-irradiated photoproduct is irreversibly formed within the prophage genome. Research on ultraviolet irradiation of replicating phage DNA at various times after prophage induction suggests that either one-third of the

replicating phage DNA is subject to excision repair or that one-third of the cellular DNA replication sites has repair capability. The exciting implication of this work is that not only do bacteria have

DNA attachment sites, some of which also have replicating capability, as is well known, but that some of the attachment-replicating sites, in addition, are capable of DNA repair.

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INTRODUCTION

During 1975, there have been several additions to the Biophysics and Instrumentation Group. Maryleu Ingram, M.D., joined the group from the Jet Propulsion Laboratory, Pasadena, California. She brings to us an extensive background in automated cytology, with an emphasis on multiparameter measurements. Her responsibilities in Group H-10 center about those aspects of our program which are related to clinical applications of the LASL-developed flow-systems instrumentation. Douglas Jackson, Ph.D., a mathematician, joined our staff in the summer of 1975. He has prime responsibility for the rather extensive PDP 11/40 and 11/45 computer systems housed in the Health Research Laboratory and used by Groups H-4, H-9, and H-10. He is responsible also for the data reduction aspects of our flow-systems biology effort.

The federally funded STEP program has provided us with two young men who are responsible for the

support aspects of the F643A and F643C programs.

These are Joseph Frank, B.S., and Richard Kissane, B.S. An additional technician, Z. V. Svitra, also joined the group in 1975.

The demands among biologists in Groups H-10 and H-9 on our instrumentation have required us to add to our staff a person to be responsible for the operation and maintenance of the four cell sorting and analysis instruments in routine use in our group. This individual is Scott McLaughlin, B.A.

The activities of the group fall into several categories: (1) development of flow-systems instrumentation for rapid cell analysis and sorting; (2) development of cell staining and preparational techniques suitable for use with flow-systems instruments; (3) biological and medical applications of these instruments; (4) physical dosimetry and radiobiology studies at the biomedical channel at LAMPF; and (5) electronic and computer support for many of the activities in the LASL biomedical program.

Specific information on each of these areas is given below.

One of the recent advances has been implementation of the drug mithramycin as a DNA stain. This chemical has been shown to be as specific for DNA as the dyes acriflavine, ethidium bromide, and propidium iodide. It has the additional advantage that the staining protocol is simple and rapid. Within 20 to 30 min after obtaining a cell sample, DNA measurements can be made using flow microfluorometry (FMF) techniques.

Our instrumentation development efforts have centered on improvements to the cell sorter, investigation of light scattering as a suitable marker, and further incorporation of computer capabilities in the cell analysis and sorting instruments.

In 1975, we have developed the capability of measuring simultaneously the light scattered by cells in a flow system at 32 angles. We have been using this system to investigate differences between cells based on the differential light-scattering patterns so measured. The advantages of the method are (1) it is non-destructive; (2) no fixation, staining, or other chemical treatment of the cells is required; and (3) because of (1) and (2), it directly answers the question of acceptability of automation in clinical cytopathology examination.

Another technique which we have pioneered is the "time-of-flight" method. In this approach, the time duration of the signal is converted to a voltage pulse proportional to the cellular dimension sweeping past the detector in the flow situation. These measurements can be accomplished either with two-color fluorescence staining or a combination of fluorescence and light scattering methods. The main areas currently under exploration include attempts to construct a nuclear-to-cytoplasmic ratio by this technique for application to clinical material.

Our pioneering work in cell analysis continues to draw national and international attention. P. F. Mullaney and H. A. Crissman were representatives at the International Workshop and Symposium in Impulse Cytophotometry at Munster, Germany, in September. Our personnel have also been invited to speak on the state-of-the-art in the flow-systems field at several National Cancer Institute symposia, including the Annual Symposium on Drug Therapy and Cell Kinetics (October 1975) and An Overview of the National Cancer Diagnosis Program, National Cancer Program (November 1975).

The flow-system approach is a very powerful tool for use in basic biology as well as clinical diagnosis. A book, "Flow Cytophotometry and Cell Sorting," by Melamed (Memorial Hospital, New York), Mullaney (LASL), and Mendelsohn (LLL), is under preparation with publication scheduled for late 1976. Several LASL staff members (G. C. Salzman, L. S. Cram, H. A. Crissman, R. A. Tobey, P. M. Kraemer, J. H. Jett, J. A. Steinkamp, P. F. Mullaney, and J. M. Crowell) are preparing chapters for this book.

During July/August 1975, a course in FMF techniques was offered by Group H-10 personnel to graduate students who participated in the J. R. Oppenheimer Summer Institute in Biophysics under the direction of J. Allred of the University of Houston.

Several of our instrumental developments have resulted in patent disclosures or patents issued: (1) fluorescent dyes for the rapid determination of cellular DNA content (S. N. 583,367), filed June 3, 1975; (2) dual parameter flow photometric apparatus and method (U. S. Patent 3,824,402), issued July 16, 1975; (3) biological cell classification by multi-angle light scattering (S. N. 583,369), filed June 3, 1975; and (4) method and apparatus for measurement of nuclear and cytoplasmic size in biological cells (S. N. 582,056), filed May 29, 1975.

DEVELOPMENT OF FLOW-SYSTEMS INSTRUMENTATION FOR RAPID CELL ANALYSIS AND SORTING

[G. C. Salzman, J. M. Crowell, J. C. Martin, J. A. Steinkamp, P. F. Mullaney, M. T. Butler, J. H. Jett, and C. A. Goad]

Most of the equipment available today for flow-systems cell analysis are zero resolution instruments, meaning that no fine structure is measured in the parameter of interest. This situation is adequate for a number of present applications; however, advances in sensitivity and resolution will permit the measurement of smaller and more weakly fluorescent objects like bacteria, algae, and cells with small concentrations of a substance of interest. Increased resolution will permit more of the morphological details of the cells to be measured. Three such approaches are considered in this report:

- (1) a time-of-flight technique which permits the nuclear and cytoplasmic dimensions to be determined;
- (2) a multiangle light-scattering photometer which permits identification of cells based on morphology;
- and (3) a 4 π light-collection system which permits a 15-fold increase in the signal collected.

Development and Application of a New Flow-System Technique for Rapid Measurement of Nuclear and Cytoplasmic Size in Mammalian Cells

The method is based on quantitative fluorescence staining of nuclear DNA with one fluorescent dye and total cellular protein with a second fluorescent dye of a different color. Nuclear and cytoplasmic size can then be measured using two-color fluorescence methods. Similarly, the nucleus can be stained only with a single-color fluorescent dye and nuclear and cytoplasmic properties measured using fluorescence and light-scatter (whole cell size) methods, respectively.

Cells stained in aqueous suspension are subsequently measured by passing them (1000/sec) in sequence through a flow chamber at a constant velocity across a narrow beam of exciting light from an argon laser. As each cell crosses the laser beam, fluorescence and light-scatter electronic signals are generated. From analysis, analog signal waveforms of DNA content, total protein, whole cell size, and nuclear and cytoplasmic diameter can be obtained. For example, DNA, protein, and whole cell size are obtained by electronic integration of the individual

signal waveforms. However, nuclear and cytoplasmic diameters are obtained by measurement of the time duration of the signals and nuclear-to-cytoplasmic diameter ratios computed. The above data are subsequently displayed as frequency distribution histograms. Mammalian cells can also be separated using high-speed electronic cell-sorting techniques.

The DNA content, total protein, light scatter (whole cell size), and nuclear and cytoplasmic properties of various cell types have been measured successfully. These include model normal and tumor cell systems cultured *in vitro*, irradiated CHO cells (x rays), and normal and abnormal human gynecological (cervical and vaginal) cell samples obtained from clinical patients. The results to date demonstrate that these important biological parameters can be measured successfully using a new technique.

Significant results of this work indicate that (1) this new and simple method lends itself to the measurement and characterization of cells based on nuclear and cytoplasmic size; (2) the technique is directly applicable to rapid screening of human gynecological cells according to the morphological criteria of nuclear-to-cytoplasmic diameter ratios, an early indication of cell malignancy; (3) the technique is directly applicable to a fairly broad spectrum of cell types; and (4) the results demonstrate a new capability for studying, establishing, and correlating DNA, protein, and nuclear and cytoplasmic diameters for both normal and abnormal cells.

A Flow-System Multiangle Light-Scattering Photometer

It is a well known principle in physical optics that the light-scattering pattern produced by an object is the Fourier transform of the morphological features of the object. Our goal is to collect a significant portion of the scattering pattern from mammalian cells so that an identification based on morphological parameters can be made. The light-scattering method does not require cell staining, an advantage for clinical applications where cytological verification is required.

The method is shown schematically in Fig. 1. Light from a helium-neon laser is focused to a 100- μ m spot in the center of the flow cell. The light-scattering detector is a photoconducting array of 32 wedges (lower portion) and 32 rings (upper portion).

Fig. 1. Schematic diagram of the multiangle light-scatter system.

In the present application, only the rings are used. Thus, the scattering pattern from 0° to 21° is measured on each cell as it passes, and this information is stored and analyzed by a PDP 11/45 computer. Essentially, the device plots the scattered light intensity as a function of angle. From past research here at LASL, we know that the forward scattering is dominated by whole cell (size) effects and that the internal structure (nucleus, etc.) contributes to the larger angle scattering. The light-scattering patterns are subjected to mathematical clustering analysis. This technique is a multivariate method which classifies the cells into types according to the variety of patterns produced.

Using this method, we have been able to classify cells based on their pattern. With human leukocytes, we have been able to detect and identify monocytes, lymphocytes, and neutrophils by measuring non-fixed, non-stained cells. Human gynecological material has also been examined. We have found three clusters with normal patients, one of which is due to leukocytes. In cases of dysplasia or invasive carcinomas, a fourth different and unique cluster appears, the nature of which has not been fully explored at this time. We are adding a sorting capability to our instrument to permit separation and verification of these cell types.

An Improved Light-Collection System for FMF Cell-Analysis Instruments

Improvement of the light-collection systems used with FMF instruments will permit measurements to be made on more weakly fluorescing cells such as algae, bacteria, or weakly stained cells (for example, small number of membrane binding sites).

Increased signal levels will also contribute to increased resolution, a necessity for measurements on complicated systems such as chromosomes.

All FMF instruments currently available as research tools or from commercial suppliers use $f/1.5$ to $f/3.0$ light-collection optics. These low numerical operative systems collect only 2 to 4% of the total light available. The most efficient light-collection system is one with a 4π geometry (i.e., all of the emitted light is collected). With our situation, such a system would permit a 20-fold increase in signal level. The method we are exploring is shown schematically in Fig. 2. Light from an argon-ion laser is brought to and focused on the cell stream through the mirror system: M_1 , M_2 , L_1 , L_2 . The flow chamber has an interior cavity which is an ellipsoid of revolution. Such a cavity has the property that all the light emitted at one focus is collected at the other focus after reflection at the elliptical surface. The chamber is arranged so that the laser beam cell stream intersection occurs at one focus, and a light guide is inserted at the second focus. The light collected then passes through a yellow laminar filter and is measured with a photomultiplier tube. At M_3 in the diagram, a mirror can be placed to permit viewing of the chamber for line-up purposes. The elliptical chamber has been coated with a gold film to provide reflection.

Fig. 2. Schematic diagram of the 4π elliptical flow chamber system.

A beam splitter has been placed in the light path which removes 4% of the laser light for monitoring. Each time a fluorescent pulse is measured, it is referenced to the laser level so that rapid variations in the laser output can be neutralized. This feature will permit a decrease in the error (dispersion) associated with FMF analysis.

The elliptical chamber has been used with test particles (plastic microspheres) and to date has produced a 15-fold increase in signal. The optical

system is difficult to align, and certain modifications are currently being made. The 15-fold signal increase is quite impressive, considering the many openings in the chamber for windows, etc., and that the reflectivity of gold is only 30% at the wavelengths we use. The system is to be optimized for chromosome measurements being made at LASL. By improving sensitivity and resolution, identification of individual chromosomes with small DNA differences should be possible.

BIOLOGICAL AND MEDICAL APPLICATIONS OF FLOW-SYSTEMS CELL ANALYSIS AND SORTING INSTRUMENTATION

[L. S. Cram, H. A. Crissman, J. A. Steinkamp, M. S. Oka, M. Ingram, and J. C. Forslund]

The flow-systems cell-analysis and sorting systems developed at the Los Alamos Scientific Laboratory provide a unique method for single-cell analysis. These devices measure each and every cell in a population at rates up to 10^3 cells/sec. By examining each cell in the population, information on subpopulations present can be obtained with statistical precision. The instruments measure optical properties of cells (fluorescence and light scatter) which can be revealed by appropriate staining of the cells. In order to exploit this instrumental method, it is necessary to establish stains specific for cellular properties of importance (membrane binding sites, virus infection, DNA content, chemical or other alteration of the cell). Hence, a significant feature of this program in the development of markers for nuclear and cytoplasmic components of the cell is explained below. This aspect of the program must be stressed before meaningful measurements can be made.

The flow microfluorometry (FMF) technique has been widely applied to basic biological studies. In a more applied vein, the method can be used as a clinical diagnostic tool for carcinogenesis, hematology, immunology, etc. Two research areas of current interest (i.e., tumor growth and immunoparticipation of monocytes) are described below. With further development of the techniques described, the methods will be applicable to monitoring cell and animal populations for chemical agents or immunological response with exposure to potentially hazardous materials related to energy production.

Spectral Characterization and Evaluation of Fluorochrome for Use in Multiple Staining and Analysis of Mammalian Cells

We plan to designate fluorochromes specific for cellular properties which can be used in a multiple staining regimen for simultaneous analysis of DNA and protein in single cells. The spectral characteristics of fluorochromes in solution and bound to cells are analyzed and evaluated on the basis of their utility in the FMF system. Specificity of the dyes for particular cellular moieties is tested, and

the FMF distributions of cells are compared with distributions obtained for cells stained with dyes of known specificity. Dyes to be used for multiple staining are evaluated on the basis of their spectral characteristics and biochemical specificity.

Rapid staining of cellular DNA can be achieved with the antitumor antibiotics mithramycin, chromomycin A_3 , and olivomycin, while the dyes rose bengal, RB 200, and SR 101 have specificity for total cellular protein. Based on the spectral properties (Fig. 1, emission spectra), the combination of olivomycin and SR 101 is feasible since the fluorescence emission of the dyes does not overlap significantly. Studies are presently in progress to determine optimal conditions for cell staining.

Multiple staining of different biochemical moieties in cells permits simultaneous analysis and correlation of those cellular properties using the multiparameter cell-analysis system. Based on the results reported previously, the analysis of both DNA and protein in single cells is feasible. The efforts here are to obtain simple and more rapid cell-staining methods.

Fig. 1. Fluorescence emission spectra of the antibiotics olivomycin and mithramycin and of the protein dyes rose bengal, lissamine rhodamine B (RB 200), and sulfarhodamine 101 (SR 101) in aqueous solution. The spectra were obtained from the Aminco-Bowman spectrophotofluorometer (American Instrument Company) using the following excitation wavelengths: 400 nm for olivomycin and mithramycin; 450 nm for RB 200 (Gurr); 400 nm for rose bengal (J. T. Baker); and 500 nm for SR 101 (Eastman Organic Chemicals). The emission spectrum for chromomycin is identical to that for mithramycin.

Analysis of DNA and Protein in Cells from Ascites and Solid Tumor Systems of Mice

The objective of this study is to characterize model tumor systems on the basis of their cellular DNA and protein content and, thus, to obtain information on the kinetics of cell proliferation and protein synthesis. Tumors obtained from mice are dispersed to provide suspensions of single cells to be stained for DNA and/or protein. FMF analysis of stained cells yields information relevant to cell-cycle progression and protein content of cells in various phases of the cell cycle.

Exponentially growing ascites cells (L1210) have DNA and protein distribution patterns (Fig. 2) similar to those observed previously with Chinese hamster (line CHO) cells grown in tissue culture. This indicates that the synthesis of both DNA and protein in L1210 cells follows a correlation pattern similar to the *in vitro* CHO system. Possibly this observation is universal for exponentially growing cells, since it is known that abnormal fluctuation in the DNA/protein ratio of cells leads to cell death. FMF analysis of solid tumors has revealed a 2C DNA content for cells from the B16 melanoma, while the Lewis lung carcinoma has a DNA content twice that of normal cells. Both solid tumors are composed of heterogeneous abnormal cell populations, and the cell types are being characterized at present. Analysis and characterization of the various tumor systems will provide more

Fig. 2. Single-parameter and complete two-parameter analysis displays of DNA and protein content of propidium iodide/fluorescein isothiocyanate stained L1210 ascites cells. The lower right-hand figure is labeled to show the various regions of the cell cycle.

information concerning normal growth conditions.

In subsequent studies, these *in vitro* systems will be used to test the chemotherapeutic value of various drug agents.

Isolation and Characterization of Human Blood Monocytes

This is a study to compare immuno-participation functions of monocytes from normal populations and populations at risk. Human peripheral blood cell suspensions containing primarily lymphocytes and monocytes are analyzed by means of an electronic cell sorter. The unique light-scatter properties of these as well as other lymphoid cells make it possible to distinguish cell types by their light-scatter properties.

Small- (1°) and large- (90°) angle light-scatter measurements have been made on lymphocytes and monocyte suspensions before monocyte depletion with carbonyl iron and plastic adherence. The left frame in Fig. 3 illustrates the light-scatter distribution from a sample containing lymphocytes and monocytes, and the right frame (B) are the same data for a monocyte-depleted sample. Monocyte populations have also been electronically sorted and the recovered cells cytochemically stained by peroxidase and nonspecific esterase tests to confirm identification as pure monocyte populations. These data serve to illustrate the excellent resolution of the two cell types by light-scatter techniques.

The functionality of monocytes in the complexities of the immune response is poorly understood due, in part, to difficulties in isolating large numbers of viable and immuno-competent cells. The role that monocytes play in addressing environmental insults is well established in the case of tumor regression in the cancer field. The findings so far indicate that greater than 90% pure and viable monocytes can be obtained by this procedure, and these cell populations will be available for characterization and determination of their role in dealing with immunological insults arising from energy production technologies.

Fig. 3. (Upper half). Two-parameter pulse-height analyzer contour map showing the number of monocytes and lymphocytes in a Ficoll-hypaque preparation before (A) and after (B) monocyte depletion by allowing the monocytes to adhere to plastic and ingest iron carbonyl before a second gradient purification. The number of cells (Z axis) is shown as a function of intensity of light scattered at 1° from the forward direction (X axis) in coincidence with that at 90° (Y axis). The larger grouping is lymphocytes. (Lower half) An isometric display of the same data.

PHYSICAL DOSIMETRY AND RADIOBIOLOGY STUDIES

[M. R. Raju, T. T. Trujillo, E. Bain, J. P. Frank, C. Richman, and J. M. Blossom]

Application of FMF to Radiobiological Problems Related to Radiotherapy

Great progress has been made in experimental physics research in the past 25 yr due mainly to development of sophisticated instrumentation. This resource, the capability of instrumentation development, resides to a large extent at the National Laboratories sponsored by the AEC during this time period. At the Los Alamos Scientific Laboratory, flow microfluorometric (FMF) techniques for the rapid measurement of mammalian cell properties have been pioneered. A benefit of this research has been the development of a method, not available previously, to yield quantitative information on each and every cell in a population. Since each cell is examined, information on small subpopulations can be obtained with high precision.

This instrumentation has many potential applications in the biological and medical sciences in studying the effects of agents such as nuclear radiation, chemicals, heat, and ultrasound on cell kinetics and cell viability. The major objective of this particular program is to study the effects of radiation and chemicals on cell progression and cell viability with experimental tumor systems. In addition, radiation treatment to spontaneous tumors in dogs is monitored by examining cells from tumor biopsies during the course of radiation therapy to determine if the measurements have any prognostic value regarding treatment success or failure.

The procedure consists of (1) preparation of single cells from the tissue of interest; (2) staining the cells with appropriate fluorescent stains specific for a particular cell property; and (3) measuring fluorescence and light-scattering properties from these cells. The work on tumor cells is in progress, and preliminary results show that the FMF technique is a powerful tool for radiobiological research. Cell viability is traditionally measured by using dyes such as trypan blue and a microscope; however, this method is subjective. Our FMF techniques can also be used to measure cell viability. Figure 1 shows the cell survival of human lymphocytes as a function of x-ray dose by

Fig. 1. Human lymphocyte cell survival as a function of x-ray dose: (-Δ-) measured by trypan blue staining using a microscope and (-●-) using the FMF technique.

(1) the trypan blue method and (2) FMF. The results indicate agreement between both methods. This FMF method has potential application in measuring cell viability in non-nuclear areas, as described previously.

Characteristics of a Negative Pion Beam for the Irradiation of Superficial Nodules in Cancer Patients

Early in 1974, the LAMPF 800-MeV proton accelerator began operation and a biomedical negative pion beam became available for preclinical studies. The philosophy that was followed was to tune the magnetic channel that guides the pion beam to produce a beam of narrow momentum interval with a maximum dose rate. The proton current in the machine reached 10 amperes with a dose rate of about 7 rads/min. This beam was eventually used to irradiate metastatic superficial nodules in four patients with systemic diseases. The follow-up of these treatments has shown some interesting and encouraging results.

To understand the nature of the beam and to prepare for the patient irradiations, an extensive series of physical studies were carried out to map the radiation field in a water phantom. The negative pion beam comes down the magnetic channel with a contamination of electrons and muons, and it is necessary to evaluate these components for the particular target and configurations used. The mean energy of the pions that enter the treatment room

is about 78 MeV. These particles have a range in tissue of about 18.8 cm. The channel also has a beryllium wedge which slows down the faster pions more than the slower pions and causes a narrowing of the momentum distribution. The resulting pions stop over a depth of 2.8 cm full width at half maximum (FWHM). The beam covers an area of about 5 cm laterally (FWHM) x 7.5 cm (FWHM). It is the purpose of the experiments to study the geometrical parameters of the beam, to identify the particles as much as possible, and to arrive at LET distributions of dose. Finally, a set of collimators was made for the irradiation of human skin nodules, and it was necessary to see how the collimators affect the various distributions. The collimators consist essentially of a nose cone made of brass with a polyethylene center to slow down the pions so that the peak dose takes place just outside the collimator. The openings for the beam are circular with diameters of 5, 4, 3, and 2 cm, all of which are smaller than the incoming beam. Therefore, one can expect that scattering of the pions will occur off the brass walls of the collimators.

The detection scheme used in this work is the lithium-drifted silicon detector. When any charged particle traverses the detector or stops in the detector, the semi-conductor faithfully yields a pulse proportional to the energy deposited. Detectors of various thicknesses were built for us by the Berkeley detector group. The areas are from 35 mm^2 to 1 cm^2 , with thicknesses of 5000, 1000, 84, 50, and 13 μm . The detectors are calibrated with radioactive sources, usually bismuth-207 (conversion electrons) and americium-241 (α -particles). Each detector is mounted in a Lucite holder (a first approximation to tissue) with a removable cap to expose the detector to short-range radiations during calibration. The electronics for amplifying and processing the pulses is a standard nuclear physics linear system. The pulses are finally analyzed by means of a Canberra 8100 pulse-height analyzer, and the spectra are then stored on magnetic tape. The tape is then used for making a detailed analysis of the spectra on our large computer.

With our arrangement of the equipment in the treatment room, the beam comes down vertically into the water phantom. The detector is mounted in a water-tight probe which is attached to the arm of a

scanner, and the pulses go to a preamplifier and then to the electronics in the counting area outside the treatment room. A separate fixed silicon probe is mounted just under the surface of the water as a beam monitor.

The pions, muons, and electrons traverse the detector, and each particle loses a certain amount of energy. This energy loss serves to identify the particle. The stars are also identified easily by the large energy release in the detector. A study of the composition of the beam using the 5000- μm thick detector shows that the incoming beam is composed of about 5% electrons, 10% muons, and 85% pions. The pulse spectra are analyzed for the different dose contributions of the various particles at different depths in the phantom. To cover the large range of energy losses that take place, a logarithmic scale is used. The results obtained with a 5000- μm -thick detector are shown in Fig. 2. The ordinate, $F(E)$, is the dose per unit logarithmic energy interval. A study of these distributions shows how the star regions build up and also the dose contributions of the various components. It is also clear from these dose measurements that the contributions due to contamination are very small.

Figure 3 is a plot of these data in terms of the low LET dose and the star dose as a function of depth in the phantom. The total dose is also shown. This total dose in silicon compares very well with the data obtained with an ionization chamber. The data show that the medium and high LET dose contained in the star is predominantly on the downstream side of the total dose distribution. This effect has also been observed radiobiologically. This kind of effect for the treatment of large tumors at various depths has to be determined for every therapeutic beam.

Figure 4 shows a similar distribution taken with a 1000- μm -thick detector under the 4-cm collimator. The star dose dominates the total dose in this region under the collimator at least for the first 2 cm. There are no large variations in relative doses immediately under the collimator.

This method of analysis determines the different kinds of doses that are important in the therapeutic process. Experiments are now in progress that utilize thin detectors (50 μm and less). With

Fig. 2. The dose per unit logarithmic energy interval, $F(E)$, is plotted vs energy at various depths in the water phantom. Development of the various doses as the beam penetrates the phantom becomes clear.

such detectors, the stars are produced predominantly in the water and not in the detector. A separation also now becomes possible of the medium LET component (protons, deuterons, and tritons) from the high LET component (α -particles, tritium, and recoils). Measurement of the various components will lead us to an understanding not only of the radiobiological effects that we can expect but also to an evaluation of a uniform dose in each of the different kinds of beams that will be used in therapy.

Fig. 3. Dose distributions in a phantom along the central axis of the beam, showing the behavior of the low LET component, the star component, and the total dose.

Fig. 4. Distributions of the low LET component and the star component along the central axis under the 4-cm collimator.

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INTRODUCTION

Stable isotopes, because of their unique properties and non-radioactive nature, have great potential for many fields of science and technology. In particular, isotopes of carbon, nitrogen, oxygen, and sulfur (the basic building blocks of all biological molecules) would be widely used in biomedical and environmental research if they were economically available in sufficient quantities and in the required chemical forms. The major objective of our program continues to be stimulation of the widespread utilization of stable isotopes and commercial involvement through development and demonstration of applications which have potential requirements for large quantities of isotopes. Thus, demand will be created which is necessary for large-scale production of stable isotopes and labeled compounds and concomitant low unit costs. The program continues to produce a variety of labeled materials needed for clinical, biomedical, chemical, and environmental applications which serve as effective demonstrations of unique and advantageous utilization of stable isotopes. Future commercial involvement should benefit, and is a consideration in our research and development, from the technology transfer that can

readily be made as a result of our organic and biochemical syntheses and also of various techniques involved in applications.

Many of our projects involving development of applications, as well as those in more fundamental research, are carried out through collaboration with investigators in other laboratories, institutions, and industries. A number of mutual interest, cooperative investigations are shared with the LASL Inorganic Chemistry Group (CNC-4), particularly those involving carbon-13 nuclear magnetic resonance spectroscopy. This LASL group also carries out production of the enriched isotopes which are, of course, absolutely essential to the entire program. Particular acknowledgment for direct contributions to several projects is made to the following Group CNC-4 personnel: N. A. Matwiyoff, biochemical and biomedical nmr investigations; R. E. London, nmr characterization of labeled cells and biosynthesized molecules; and B. B. McInteer and T. Mills, mass spectrometric isotope ratio determinations for clinical breath samples and other products.

Basic photosynthetic processes are being conducted in collaboration with the Laser Research and Technology Division and the Theoretical Division

through the LASL's New Research Initiatives Program.

Initial funding by the Division of Research Resources, National Institutes of Health, for a "National, Stable Isotopes Resource at Los Alamos" (N. A. Matwiyoff, CNC-4, principal investigator) has been obtained. In addition to contributing to NIH objectives, it will increase and broaden our capabilities and thus be beneficial to presently established goals as well.

In the coming year, it is anticipated that increased attention will be given to Group E-11's existing and potential capabilities for contributing to solutions of environmental problems and other aspects of developing energy programs (e.g., solar energy utilization through photosynthesis and bio-conversion).

NON-RADIOACTIVE BIOMEDICAL AND ENVIRONMENTAL TRACERS: STABLE ISOTOPES

Any material which is present at an abnormal concentration compared to that usually found can serve as a "tracer" in following the movements of the system with which it is associated. "Tagging" with fluorescent dyes or radioactive isotopes is a well-known example. Certain naturally occurring, stable isotopes of various elements also have great potential for application in many fields of science and technology because of their non-radioactive nature and unique physical properties. In particular, isotopes of carbon, nitrogen, and oxygen (basic building blocks of all biological molecules) would be widely applicable in biological, medical, and environmental studies if they were readily available in the required chemical forms at reasonable cost. A program to stimulate and develop the applications of stable isotopes, which will create significantly greater demand resulting in greatly increased material production levels and consequent reduction of the cost-use barrier, has been in progress for the past several years.

This problem is dependent on one also established for large-scale production of the isotopes (i.e., separation and concentration of the naturally occurring nuclides, carbon-12, carbon-13, nitrogen-14, nitrogen-15, oxygen-16, oxygen-17, and oxygen-18). Before the isotopes can be utilized, however,

they must (in most cases) be incorporated into organic molecules which are more complex than the simple chemical forms in which they are separated (carbon monoxide and nitric oxide). Preparation of a wide variety of labeled compounds has been made through the development of efficient methods of organic and biochemical syntheses. New procedures must be devised frequently owing to the nature and cost of the available starting materials. In addition to providing the labeled compounds required for development of particular applications, the synthetic procedures also become available for future use by other investigators in all laboratories and for commercial production by private industry when sufficient demand makes this possible. Such continuing technology transfer is extremely important to the wide-scale applications of stable isotopes.

Key synthetic intermediates are relatively simple compounds which (in addition to often being useful per se) are necessary for the preparation of more complex molecules (Fig. 1). Each compound prepared paves the way for additional useful intermediates and products. All organic syntheses originate with the very simple molecule, carbon-¹³C monoxide, which is the product from the isotope enrichment facility. Utilization of living organisms for biosynthesis of labeled compounds often provides the most efficient route, particularly for complex material which would be extremely difficult

Fig. 1. Key synthetic intermediates.

TABLE I

TYPES AND EXAMPLES OF BIOSYNTHETIC LABELING

SELECTIVEUniform

Glucose from carbon dioxide (green plant leaf photosynthesis)

Lactate from glucose (*L. delbrueckii* fermentation)

Lysine from glucose (*M. glutamicus* mutant fermentation)

Specific

Cytochrome c from histidine (*H. crassa* mutant culture)

Tryptophan from indole (*C. purpurea* fermentation)

TOTAL (UNIFORM)

Algae from carbon dioxide and/or ammonia

Yeast from acetate

Green plants from carbon dioxide/ammonia

by the methods of organic chemistry. In certain cases, combination of organic and biochemical syntheses is advantageous (Table I).

Numerous applications in many fields of science and technology have been stimulated and developed through collaboration with investigators in other laboratories and institutions. It is anticipated that many of these will become generally applicable in the near future as the utility of the methods is realized and the associated techniques and instrumentation are acquired by additional researchers and technologists. Stable isotopes will be quite advantageous for certain non-invasive clinical diagnostic procedures, particularly in pregnant women, children, and large population screening

where the use of radioactive isotopes is contraindicated. Certain clinical trials now in progress appear very promising (e.g., for fat malabsorption, errors in carbohydrate metabolism, and liver dysfunction). These tests depend on the relatively simple determination of the $^{13}\text{C}/^{12}\text{C}$ ratios in the exhaled carbon dioxide of the patient's breath following oral administration of the proper labeled compound prepared by organic or biochemical synthesis. In order to reassure the diagnosticians, as well as to acquire fundamental information about certain biological systems, possible biological isotope effects are being looked for in a variety of organisms, including mammalian. As expected, no effects of increased isotope concentration, even at very high levels, have been observed which would hinder biological and medical applications of these isotopes.

The use of isotopically labeled hydrocarbons to study atmospheric transport and diffusion on a continental scale has been demonstrated by the release of methane- $^{13}\text{C}-\text{d}_4$ and subsequent detection in air samples at distances of 1500 to 2500 km from the point of release. The small amounts needed and the non-toxicity of such stable isotope-labeled tracers should make possible their use even in congested urban areas. Tracing of various atmospheric and ecological pollutants by specifically "fingerprinting" them through unique multiple-isotope labeling is expected to become a very useful technique for assessing the movements and fate of materials associated with energy production and industrial and agricultural practices.

APPENDIX A

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APPENDIX B

SYMPOSIA

WORKSHOP ON SOMATIC CELL GENETICS. I. "Mutant Isolation and Characterization"

May 28-30, 1975

Sponsored by the Health Division of the Los Alamos Scientific Laboratory and the Division of Biomedical and Environmental Research of the U. S. Energy Research and Development Administration

Wednesday, May 28, 1975

"ERDA and Somatic Cell Genetics," Discussion with ERDA Representatives

Thursday, May 29, 1975

Welcome

D. F. Petersen, Alternate Health Division
Leader, Los Alamos Scientific Laboratory

"Automation of Petri Dish Technique"

D. A. Glaser, University of California,
Berkeley

"Quantitative Analysis for Mutations at the HGPRT
Locus Induced by Physical and Chemical Agents in
CHO Cells"

A. W. Hsie, Holifield National Laboratory, Oak
Ridge

"Conversion of Chemical Carcinogens into Active
Mutagens by Liver Extracts"

T. S. Matney, University of Texas Health
Science Center, Houston

"Induction, Isolation, and Segregation of Co-
Dominant Mutations in Mammalian Somatic Cells"

M. J. Siciliano and R. M. Humphrey, Univer-
sity of Texas Cancer Center, Houston

"Genetics of Somatic Cell Surface Antigens"

C. A. Jones, Eleanor Roosevelt Institute for
Cancer Research, Denver

"Isolation and Characterization of Cell Cycle
Mutants in a Near-Diploid Murine Cell Line"

M. Liskay, University of Colorado, Boulder

"Selection and Characterization of Adhesion Mutants
of CHO Cells"

A. G. Atherly, B. J. Barnhart, P. M. Kraemer,
and R. T. Okinaka, Los Alamos Scientific Lab-
oratory

"Temperature Sensitive Mutants of BHK21 Cells"

H. Meiss, New York University School of Med-
icine, New York City

"Temperature Sensitive Mutations Affecting Protein
Synthesis in CHO Cells"

L. Thompson, Lawrence Livermore Laboratory

"Thymidine Requiring Mutants of CHO-K1 and Carbo-
hydrate Utilization by CHO-K1, V79, and CHS"

M. L. Morse, Webb-Waring Lung Institute,
Denver

"Selection of Temperature Sensitive Mammalian Cell
Mutants Defective in Macromolecular Biosynthetic
Processes"

D. J. Roufa, Baylor College of Medicine,
Houston

Friday, May 30, 1975

"Genetic Analysis with Auxotrophic Mutants"

F.-T. Kao, Eleanor Roosevelt Institute for
Cancer Research, Denver

"A Search for Mitotic Recombination in Cultured
Chinese Hamster Cells"

L. Chasin, Columbia University, New York City
"Spontaneous and Mutagen Induced Segregation Events
in Hybrid CHO Cells"

J. Harris, Ontario Cancer Institute, Toronto
"Parasexual Approaches to Gene Localization and
Regulation"

F. Ruddle, Yale University, New Haven
"Selection and Application of Mutants in Higher
Plant Cells"

T. B. Rice, Michigan State University, East
Lansing

"Haploid Frog Cell Variants and Mutagenesis"

J. J. Freed, Institute for Cancer Research,
Philadelphia

"Summary-Overview"

F. Ruddle, Yale University, New Haven

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LASL THIRD LIFE SCIENCES SYMPOSIUM

"Impact of Energy Production on Human Health"
An Evaluation of Means for Assessment

October 15-17, 1975

Sponsored by the Health Division of the Los Alamos
Scientific Laboratory and the Division of Biomedical
and Environmental Research of the U. S. Energy
Research and Development Administration

Wednesday, October 15, 1975

Welcome

G. L. Voelz, M.D., Health Division Leader,
Los Alamos Scientific Laboratory

SESSION I: OVERVIEWS OF THE PROBLEMS

Chairman, G. R. Shepherd, Division of Bio-
medical and Environmental Research, U. S.
Energy Research and Development Administration

"An Overview of Our National Energy Needs, Resources, and Future Developments"

R. W. Fri, Deputy Administrator, U. S. Energy Research and Development Administration

"An Overview of Responsibilities and Capabilities Related to Health Impacts of Energy Production"

J. L. Liverman, Assistant Administrator for Environment and Safety, U. S. Energy Research and Development Administration

"Health Hazards Associated with Alternate Energy Sources"

D. P. Rall, Director, National Institute of Environmental Health Sciences

SESSION II: REVIEW OF STANDARDS SETTING

Chairman, C. R. Richmond, Associate Director for Biomedical and Environmental Sciences, Holifield National Laboratory, Oak Ridge

"Setting Standards for the Worker: An Historical Perspective"

E. J. Baier, Deputy Director, National Institute for Occupational Safety and Health

"Setting Standards for the Public: An Historical Perspective"

M. Corn, Graduate School of Public Health, University of Pittsburgh

"Principles of Standards Setting: The Ionizing Radiation Experience"

H. M. Parker, HMP Associates, Inc.

"Developing Health Standards: Responsibility of the Scientist in Developing and Interpreting the 'Public Interest' Viewpoint"

S. S. Epstein, School of Medicine, Case Western Reserve University

Thursday, October 16, 1975

SESSION III: SOURCES OF INFORMATION

Chairman, D. F. Petersen, Alternate Health Division Leader, Los Alamos Scientific Laboratory

"Developing a Health Standard from Epidemiological and Clinical Data"

J. H. Knelson, Director, Human Studies Laboratory, National Environmental Research Center, U. S. Environmental Protection Agency

"Developing a Health Standard from Laboratory Animal Data"

L. B. Tepper, Commissioner of Science, Food and Drug Administration

"Carcinogenesis as an End Point in Health Impact Assessment"

M. Schneiderman, Field Studies and Statistics, National Cancer Institute

"Mutagenesis and Teratogenesis as End Points in Health Impact Assessment"

M. A. Bender, Medical Department, Brookhaven National Laboratory

SESSION IV: SOURCES OF INFORMATION (continued)

Chairman, P. P. Cohen, Department of Physiological Chemistry, University of Wisconsin Medical School

"Behavioral Changes as End Points in Health Impact Assessment"

B. Weiss, School of Medicine and Dentistry, University of Rochester

"New Methods and Approaches to Health Impact Assessment"

M. L. Mendelsohn, Associate Director, Lawrence Livermore Laboratory

"Problems in Sampling for Health Impact Assessment"

A. P. Altshuller, Division of Chemistry and Physics, U. S. Environmental Protection Agency

"The Applications of Scientific Data to Decision-Making"

W. T. Brooks, Organization Resources Counselors, Inc.

Friday, October 17, 1975

SESSION V: SPECIAL PROBLEMS

Chairman, A. Wolman, Emeritus Professor, The Johns Hopkins University

"Bases for the Application of Scientific Data to Health Protection"

J. W. Healy, Assistant Health Division Leader, Los Alamos Scientific Laboratory

"Health Standards: A Legal Point of View"

R. V. Zener, General Counsel, U. S. Environmental Protection Agency

Discussion Period: Room for Controversy

Moderator, S. M. Greenfield, Greenfield, Attaway & Tyler, Inc.

Panel: L. R. Valcovic, National Institute of Environmental Health Sciences

S. S. Epstein, Case Western Reserve University

J. W. Healy, Los Alamos Scientific Laboratory

J. H. Knelson, U. S. Environmental Protection Agency

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